The Natural History of Pregnancy Loss

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Submitted in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy
under the Executive Committee
of the Graduate School of Arts and Sciences

COLUMBIA UNIVERSITY

2016

ABSTRACT

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Pregnancy loss, the demise of a pregnancy at any time between implantation and delivery, is a common event in women's lives, affecting approximately one in three pregnancies. Pregnancy loss often causes profound psychological distress to women, their partners, and their families. However, despite its frequency and troubling nature, relatively little is known about the natural history of pregnancy loss, especially the multitude of signs and symptoms that precede a loss and distinguish it from an ongoing healthy pregnancy. One of the challenges in describing the natural history of pregnancy loss is that most losses occur very early, before entry to clinical care, necessitating the use of preconception cohort studies. Few such studies have ever been conducted worldwide. This dissertation aimed to describe the natural history of early pregnancy loss at <20 weeks gestation for the first time using a unique preconception cohort with daily prospective follow-up from the start of the pregnancy attempt through seven weeks post-conception.

To accomplish this goal, three specific aims were undertaken. First, a systematic literature review was conducted to synthesize the existing literature on the relationships between the signs and symptoms and pregnancy loss. Two analytic aims were then undertaken to delineate thoroughly the relationships between prospectively ascertained signs and symptoms—namely, vaginal bleeding, lower abdominal cramping, nausea and vomiting (hereafter referred to as

"signs and symptoms")—and subsequent early pregnancy loss. The first analytic aim used a fixed covariate and fixed effect survival analytic approach to estimate the cumulative incidence of early pregnancy loss by the presence of individual, combinations, and patterns of signs and symptoms and the associations between signs and symptoms and the cumulative incidence of pregnancy loss. The second analytic aim used a time-varying covariate and time-varying effect survival analytic approach to estimate the weekly associations between signs and symptoms and pregnancy loss to determine if these relationships were consistent or divergent across gestational ages. The results of the first and second analytic aims were then compared to gain a more complete understanding of the natural history of early pregnancy loss.

The literature review revealed a dearth of studies on the signs and symptoms of pregnancy loss. Two preconception and 16 pregnancy cohort studies were identified. The literature suggested that vaginal bleeding, particularly heavy vaginal bleeding, was associated with an increased risk of pregnancy loss while vomiting, and in some studies nausea, was associated with a decreased risk of pregnancy loss. However, reliance on care-seeking cohorts, maternal retrospective reports of signs and symptoms after pregnancy loss, and retrospective recall of signs and symptoms over long periods (e.g., entire trimesters) may have biased the observed associations between signs and symptoms and pregnancy loss leading to incorrect inferences regarding the relationships between signs and symptoms and pregnancy loss.

The two analytic aims addressed the data gaps identified in the literature review. The preconception cohort design with prospective daily follow-up from the beginning of the

pregnancy attempt facilitated the ascertainment of pregnancies at the earliest stages of gestation and losses prior to clinical care entry through the use of urine-based home pregnancy testing. The daily reporting of multiple signs and symptoms in the first five weeks after a positive home pregnancy test, or approximately two to seven weeks post-conception, allowed for a full description of the relationships between signs and symptoms of pregnancy loss without recall bias.

Data for the two analytic aims come from the Longitudinal Investigation of Fertility and the Environment (LIFE) Study, a population-based cohort with preconception recruitment of couples in 16 counties in Michigan and Texas followed for 12 months of trying for pregnancy and then through pregnancy loss or delivery for couples achieving an hCG pregnancy. 501 couples entered the study, and 347 achieved a pregnancy during the study period. Three hundred forty-one singleton pregnancies comprise the study population for the two analytic aims in this dissertation. Overall, 95 (28%) pregnancies in the study population ended in a pregnancy loss. Lower abdominal cramping, nausea, and vomiting were often reported during the early pregnancy period; vaginal bleeding was less common. The results of the fixed covariate fixed effect survival analysis from the first analytic aim demonstrated that vaginal bleeding, particularly heavy bleeding and bleeding accompanied by lower abdominal cramping, was associated with an increased risk of pregnancy loss. In contrast, the presence of vomiting, but not nausea alone, during the early pregnancy period was associated with a lower risk of loss. Analyses in the second analytic aim using weekly time-varying covariates and time-varying effects of signs and symptoms on pregnancy loss revealed some new findings. The first week after a positive pregnancy test appeared to be a vulnerable period. Vaginal bleeding and lower

abdominal cramping were associated with an increased risk of loss in the first week but not in later weeks; conversely, nausea and/or vomiting were associated with lower risk of pregnancy loss but only after the first week.

The observed weekly variations in the signs and symptoms of pregnancy loss may reflect changes in maternal adaptation to pregnancy across gestation. Overall, relatively little is known about the biological processes underlying healthy and unhealthy adaption to pregnancy as well as how embryo quality may affect these adaptive processes. More work is required from basic scientists, clinicians and epidemiologists to better understand the causes of signs and symptoms and their relationships to pregnancy loss, including genetic and environmental factors and their interactions. In the meantime, prognostic models developed from data in this dissertation using time-varying signs and symptoms may be useful to women and their health care providers for identifying pregnancies at increased risk for pregnancy loss. These models could prompt women to seek medical care when concerning patterns of signs and symptoms arise.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

CHAPTER 2

CHAPTER 3

ACKNOWLEDGEMENTS

Thank you to my dissertation sponsor, Dr. Cande Ananth, for your abiding confidence in me; your guidance and encouragement has facilitated my success and I am deeply grateful. Thank you to my NICHD mentor, Dr. Germaine Louis, for setting me on this path by bringing me to NICHD, introducing me to pregnancy loss, and allowing me use of the LIFE Study data. Thank you to my other dissertation committee members, Drs. Sandro Galea, KS Joseph, and Lisa Bates, for challenging and motivating me. Thank you to Dr. Rajeshwari Sundaram for helping me better understand survival analysis and making me smile on difficult days. Thank you to Drs. Leslie Davidson, Adam Ratner, and Shari Gelber for championing me and mentoring me early in my doctorate program; you set the stage for my success. Thank you to Liliane Zaretsky for a shoulder to cry on innumerable times; your friendship is priceless. Among many others, thank you to Drs. Kirsten Lum, Ling Ma, Melissa Smarr, Ashok Chaurasia, Jared Foster, and Adina Lemeshow for reassurance from the other side of the PhD divide, and to my Columbia cohort for your assistance during coursework and exams. To my family, I am eternally grateful. Thank you to my mother, Kimberly Kiernan, for inspiring me to pursue big dreams and sacrificing to ensure I achieved them. Thank you to my father, James Hensel, for believing in my dreams and reminding me I am capable of realizing them. Thank you to my younger siblings, Victoria, Mark, and Elizabeth Hensel, for being there whenever I need a laugh or a cry; you all motivate me to persevere. Thank you to my family by marriage, Jagjit, Anita, Mrinalini, and Dr. Sonalini Sapra, for your unwavering belief in me, and to Van Morrison for being my constant canine companion. Finally, most importantly, thank you to my husband, Dr. Eshanjit Sapra, for supporting and encouraging me throughout this most arduous process, for reminding me I am stronger and smarter than I think I am, and for telling me everything will be alright; you were right.

DEDICATION

To women around the world,

Who are at greater risk of death, violence, and ill-health simply because of your gender,

When you find yourselves in darkness, may you also find a light to guide you out;

To future generations of women,

May the efforts of those of us on Earth today

Brighten the light and vanquish the darkness for you;

and

To Eshanjit,

You are the light in my life,

You have led me out of the darkness countless times,

Thank you,

Main tumse pyaar karti hoon.

CHAPTER 1: INTRODUCTION

Pregnancy loss, from implantation through delivery, is a common, often upsetting, and poorly understood event in women's lives. Estimates of the cumulative incidence of pregnancy loss from prospective preconception studies vary widely, though many studies report one loss in every three to four pregnancies.[1-21] Most losses occur very early in pregnancy, prior to clinical care entry, and are often only detected by sensitive tests for human chorionic gonadotropin (hCG).[1,2,4,8,9,13,14,16,18,19,22] Despite the frequency of pregnancy loss, its natural history, and the temporal ordering of multiple signs and symptoms in relation to loss, has yet to be fully delineated. Data from prospective preconception cohort studies are necessary in order to assess the relationship between specific signs and symptoms and early pregnancy loss. However, such data are limited due to the cost and complexity of implementing preconception cohort studies. The goal of the literature review was to identify and summarize the existing literature to provide the context and motivation for the two analytic aims.

Specific Aim 1. To conduct a comprehensive literature review on the associations between signs and symptoms and pregnancy loss. Due to the dearth of literature on the signs and symptoms associated with pregnancy loss, I included findings from preconception and prospective pregnancy cohort studies. This review summarized the existing literature, highlighting the critical data gaps in the natural history of pregnancy loss, and included some discussion on the putative mechanisms by which signs and symptoms may precede a pregnancy loss. This review provided the context and motivation for analytic Aims 2 and 3 in this dissertation.

In the context of limited prospective data on signs and symptoms of pregnancy loss, the overarching goal of the two analytic aims of this dissertation was to describe the natural history of pregnancy loss <20 weeks gestation by delineating the temporal ordering of signs and symptoms during 4-9 weeks gestation in relation to early pregnancy loss using data from the Longitudinal Investigation of Fertility and the Environment (LIFE) Study, a prospective preconception, population-based cohort of 501 couples attempting pregnancy in Michigan and Texas, USA. To my knowledge, the LIFE Study was the first to collect daily data on multiple signs and symptoms during the early pregnancy period.

The two analytic approaches for Aims 2 and 3 were chosen because they addressed different, but complimentary, questions using available data from the LIFE Study. Using a fixed covariate, fixed effect approach in Aim 2, I answered the question about the average effect (i.e., marginal means) of individual and combinations of signs and symptoms occurring from ~4-9 weeks gestation on early pregnancy loss. Using a time-varying covariate, time-varying effect approach in Aim 3, I answered the question about the week-specific effects of individual and combinations of signs and symptoms occurring from ~4-9 weeks gestation on early pregnancy loss and whether these relationships change across gestation. Taken together, the results from these two aims provided information about average and week-specific effects of signs and symptoms on early pregnancy loss. They also provided information on the effects of shorter (weekly) and longer (up to five weeks) windows of exposure to signs and symptoms on early pregnancy loss.

Specific Aim 2. To delineate the natural history of pregnancy loss as captured by the daily reporting of possible signs and symptoms of loss from two to seven weeks post-conception. The incidence of signs and symptoms among women experiencing pregnancy loss was described and contrasted with the incidence among women not experiencing a loss. Pregnancy loss included losses <20 weeks gestation, exclusive of ectopic pregnancies. Signs and symptoms were included as fixed covariates with fixed effects on pregnancy loss across gestational age in survival analytic models. Signs and symptoms included bleeding, lower abdominal cramping, and nausea and/or vomiting. Individual signs and symptoms, combinations of signs and symptoms, and temporal patterning of signs and symptoms in relation to pregnancy loss were evaluated.

Specific Aim 3. To determine whether the associations between signs and symptoms and pregnancy loss were modified by gestational age at loss. Survival models similar to those in Aim 2 were constructed with two important differences: the signs and symptoms and the effects of signs and symptoms were allowed to vary across gestational ages. By allowing the associations between signs and symptoms and pregnancy loss to vary, I was able to determine if the relationships between signs and symptoms and pregnancy loss were modified by gestational age. Results from the time-varying effect survival models were compared with the results from the fixed effect survival models in Aim 2.

CHAPTER 2: SIGNS AND SYMPTOMS OF PREGNANCY LOSS: A SYSTEMATIC REVIEW

ABSTRACT

Approximately one-third of pregnancies end in a loss; however, the natural history of early pregnancy loss, including signs and symptoms preceding a loss, has yet to be fully described. I searched PubMed/MEDLINE and Embase to identify articles with prospective ascertainment of signs and symptoms of pregnancy loss <20 weeks gestation in spontaneous conceptions with a focus on vaginal bleeding, nausea, and vomiting. Two preconception and 16 pregnancy cohort studies that attempted to ascertain information on bleeding and/or nausea/vomiting prior to the ascertainment of pregnancy loss were included. Data from these studies indicated that vaginal bleeding was associated with an increased risk of loss, while nausea and vomiting were associated with a decreased risk of loss, though these studies were mostly comprised of pregnancies surviving into the late first trimester. While such associations were biologically plausible, these study designs were subject to bias given recruitment of women at later gestational ages and reliance on women presenting to care and reporting symptoms to their clinicians. Furthermore, the details of reporting across studies varied greatly with only one study collecting daily data on bleeding. Unstructured reporting (e.g., unprompted reporting to clinicians) or reporting over long periods (e.g., monthly) may have introduced reporting error and bias. Data gaps remain regarding 1) relationships between signs and symptoms and losses occurring very early, prior to care entry, 2) patterns of the different signs and symptoms preceding pregnancy loss, and 3) empirical testing of whether relationships between signs and symptoms and loss differ across gestational age.

INTRODUCTION

Pregnancy loss is the spontaneous end of a pregnancy resulting in embryonic or fetal demise at any point from implantation through delivery. Pregnancy loss affects approximately one-third of pregnancies and most often occurs before viability during the first and early second trimesters.[4,14] Pregnancy loss is often an upsetting event for both women and their partners.[23-28] Despite the frequency and potentially distressing nature of pregnancy loss, the pathophysiology of such loss remains poorly understood, and its natural history, including temporal ordering of signs (objective findings by clinician or patient) and symptoms (subjective patient experience) in early pregnancy has yet to be fully described.

The signs and symptoms of pregnancy and loss most often evaluated in epidemiologic studies include nausea, vomiting, and vaginal bleeding with or without associated pain and/or cramping. Nausea and vomiting are believed to be protective against pregnancy loss while bleeding, pain, and cramping are believed to be more ominous. Given the need to more thoroughly delineate the signs and symptoms of pregnancy loss, the objectives of this review were 1) to determine the state of existing knowledge on signs and symptoms of early pregnancy loss (<20 weeks gestation) with regards to the incidence of signs and symptoms and the cumulative incidence of early pregnancy loss in women with and without signs and symptoms and 2) to identify any data gaps, particularly with regard to populations studied (care-seeking women versus all women).

METHODS

Literature search

I conducted a PubMed/MEDLINE search using search parameters listed in Table 2.1.A. Searches for abortion, spontaneous (MeSH Term) and miscarriage (MeSH Term) yielded the same results. I also conducted an Embase search using search parameters listed in Table 2.1.B. Reference lists of all included papers were also crosschecked, and the reference lists of prior review papers on bleeding[29,30] or nausea and/or vomiting of pregnancy (NVP)[31,32] were searched. No restrictions were placed on publication date. Only articles written in English were included. I completed all searches and data extraction; the last search was completed November 19, 2015.

Inclusion/exclusion criteria

Preconception studies are ideal for evaluating the relationships between signs and symptoms and early pregnancy loss as they can capture all pregnancies detectable by available technology (e.g., highly sensitive home pregnancy tests), and they do not depend upon pregnancies surviving until clinical detection. However, due to a dearth of preconception studies on signs and symptoms associated with pregnancy loss, I also included prospective cohort studies recruiting women during pregnancy. I excluded studies focused on couples seeking infertility treatment, women with recurrent pregnancy loss, ectopic pregnancy, molar pregnancy, twin pregnancy, antepartum hemorrhage, subchorionic hematoma, hyperemesis gravidarum or existing medical conditions, studies with report of symptoms exclusively after pregnancy loss (including case-control

studies), studies where prescription of antiemetic drugs was used as proxy for vomiting, studies where indication for ultrasound or chief complaint for emergency department presentation were used as proxies for bleeding, studies without a comparison group or an inappropriate comparison group (e.g., ectopic pregnancies), studies focused on prediction of viability using ultrasound or biological markers, studies on treatments for nausea and vomiting, studies on stillbirth, preterm birth or other adverse pregnancy outcomes, and studies without data on pregnancy outcomes. Studies on threatened abortion were only included if they compared loss rates in women with and without other signs and symptoms (e.g., nausea and/or vomiting). Matched cohort study designs were excluded as incidence of signs and symptoms could not be estimated. Cross-sectional studies were excluded since the outcomes for all pregnancies were not known at the time of the study's end. Abstracts were read to ensure articles met the inclusion/exclusion criteria; if ambiguous, the full manuscript was read to ascertain if it merited inclusion in my review.

Data synthesis

Given the paucity of data from preconception studies, my synthesis considered both preconception and pregnancy cohort studies together despite selection and reporting biases inherent in this design (discussed in the *Limitations of existing literature on signs and symptoms of pregnancy loss* subsection below). The cumulative incidence of each sign and symptom as well as the cumulative incidence of pregnancy loss among women experiencing and not experiencing specific signs and symptoms were reported. Risk of pregnancy loss in women with signs and symptoms was compared with risk of loss in women without signs and symptoms

using data abstracted from the articles to estimate risk ratios (RR) and 95% confidence intervals (CI). No meta-analysis was undertaken given the relatively small number of eligible studies.

RESULTS

Figure 2.1 shows the number of articles identified, excluded, and included in the final review. The literature search yielded 19,550 articles of which 4,187 were duplicates leaving 16,804 unique titles. 112 articles passed the title review and their abstracts were read. 45 articles passed the abstract review and the full-text was read for suitability of inclusion in the review. Of these articles, 29 were excluded for the following reasons: indication for ultrasound proxy (n=6), chief complaint proxy (n=3), no comparison group (n=5), inappropriate comparison group (n=3), other pregnancy outcome (n=4), antepartum hemorrhage (n=1), other signs or symptoms (n=1), retrospective report (n=1), biomarker prediction study (n=2), matched cohort study (n=1), and cross-sectional study (n=2). Two additional studies were identified from previous systematic reviews and reference lists of included articles. In total, 18 studies were included in the review on the incidence of signs and symptoms and associations with pregnancy loss <20 weeks gestation. Two studies were preconception cohorts, and 16 studies were pregnancy cohorts.

Cumulative incidence of vaginal bleeding and its associations with pregnancy loss

Care-seeking cohorts

Four prospective studies on vaginal bleeding and its association with pregnancy loss from cohorts of women seeking prenatal care were included (Table 2.2).[33-36] The studies were

conducted from the 1960s into the 2000s in three different countries, and sample sizes ranged from 550 patients in a general practice to >16,000 patients in a multicenter trial for trisomy 21 screening. The incidence of vaginal bleeding in pregnancy ranged from 7-21%, with the wide range likely reflecting the varying extent to which bleeding was captured in medical charts, which depended upon (1) gestational age at care-seeking, (2) women reporting bleeding, and (3) clinicians recording the reports. The incidence of loss in women with bleeding ranged from 1-56%, while the incidence of loss among women without bleeding ranged from <1-7% of women. This resulted in RRs of 2.6 (95% CI: 1.3, 5.2),[33] 2.8 (95% CI: 1.7, 4.4),[36] 8.6 (95% CI: 6.6, 11.2),[35] and 120 (95% CI: 30, 484)[34] in the four studies. In one study reporting on severity of bleeding, heavy bleeding carried a greater risk of loss relative to no bleeding (RR 4.9, 95% CI: 2.0, 12.2) compared with light bleeding relative to no bleeding (RR 2.5, 95% CI: 1.5, 4.1).[36]

Community-based cohorts

Two prospective cohort studies of female volunteers recruited from the community were included (Table 2.2).[37,38] In one US preconception cohort of 151 pregnancies, the cumulative incidence of bleeding ≤8 weeks gestational age among pregnancies surviving ≥6 weeks gestational age was 9%.[38] Only 15 pregnancy losses were recorded, with 2 occurring among 14 women with bleeding (14%) and 13 occurring among 137 women without bleeding (9%), yielding an RR of 1.5 (95% CI: 0.4, 6.0) for bleeding. In a US pregnancy cohort of 4,510 pregnancies, the cumulative incidence of retrospectively reported first trimester bleeding was 27% with 8% reporting heavy bleeding.[37] Cumulative incidence of loss was 11% in women with any bleeding, 24% in women with heavy bleeding, and 12% in women without any bleeding.

Any bleeding versus no bleeding was not associated with pregnancy loss (RR 0.9, 95% CI: 0.8, 1.1); however, heavy bleeding was associated with increased risk of pregnancy loss (RR 2.0, 95% CI: 1.4, 3.0); heavy bleeding accompanied by pain was associated with the highest risk of loss (RR 3.1, 95% CI: 2.1, 4.5). Longer duration of bleeding was also noted to increase the risk of pregnancy loss. Though not statistically evaluated, it was also observed that the weekly probability of loss among women with bleeding was greater earlier in gestation.

Cumulative incidence of nausea and vomiting and its associations with pregnancy loss

Care-seeking cohorts

Nine prospective studies of NVP and its associations with pregnancy loss among cohorts of women seeking prenatal care were included,[39-47] spanning four countries and 50 years (four had sample sizes >1000 patients, Table 2.3). These included studies from a large insurance provider,[39,46] the multicenter Collaborative Perinatal Project,[40] and a study of women seeking prenatal care in Malmo, Sweden.[47] Incidence of NVP prior to 20 weeks gestation ranged from 65-89%. Incidence of loss among women with NVP (range 0-11%) was lower than the incidence of loss among women without NVP (range 7-35%), resulting in RRs ranging from 0.2-0.6.

Several studies have reported the cumulative incidence of vomiting separately.[40,43-45] The incidence of vomiting ranged from 46-56%. The cumulative incidence of loss was consistently lower among women with vomiting (range 1-5%) than among women with nausea alone (range

4-10%). The RRs for vomiting compared with no NVP ranged from 0.1-0.6 whereas the RRs for nausea alone compared with no NVP ranged from 0.5-0.7.

Community-based cohorts

Two prospective studies on NVP and its associations with pregnancy loss among cohorts of female volunteers recruited from the community were included (Table 2.3).[48,49] One was a US preconception study of 585 pregnancies with monthly reporting of nausea allowing for reporting after a loss.[49] Eighty-eight percent of women reported first trimester nausea; 7% of women with nausea had a loss compared with 30% in women without first trimester nausea (RR 0.2, 95% CI: 0.2, 0.4). In a US pregnancy cohort of 2,407 pregnancies with first trimester recruitment, 89% reported NVP in first or second trimesters; 53% reported vomiting.[48] Odds of loss were greater in women without NVP compared with any NVP (odds ratio 5.7, 95% CI: 4.0, 8.0). Odds of loss were also greater in women with nausea only compared with vomiting (odds ratio 2.4, 95% CI: 1.8, 3.3).

Patterning of signs and symptoms of pregnancy loss

Evidence from clinical reports in the 1950s suggested some combinations of signs and symptoms may portend pregnancy loss. Speert and Guttmacher[42] noted that among 31 patients with a first trimester loss, three-quarters had no NVP whereas among 225 women who did not experience loss, including 49 who reported bleeding, 70% reported some NVP. They concluded that heavier, darker bleeding accompanied by cramping in the absence of nausea likely signaled impending

loss. Medalie[41] also noted the protective association of NVP against loss in the setting of bleeding. Among 23 women with bleeding, one woman reported moderate/severe NVP and she did not experience a loss while 50% of women reporting no/mild NVP did experience loss (Table 2.4). More recently, among a series of women presenting for threatened abortion between 5-10 weeks gestation who were followed through 16 weeks gestational age, women who reported nausea during pregnancy were less likely to experience loss than women without nausea (hazard ratio 0.3, 95% CI: 0.1, 0.6).[50]

DISCUSSION

Main findings

Data from prospective studies, mostly conducted among care-seeking populations recruited during pregnancy, suggested that vaginal bleeding was associated with increased risk of pregnancy loss, while nausea and vomiting were inversely associated with pregnancy loss. However, there were several potential biases inherent in these study designs, namely, length-biased sampling (selective inclusion of late pregnancy losses, see *Limitations of existing literature on signs and symptoms of pregnancy loss* subsection below), recall bias (reporting of signs and symptoms after a loss), and under-ascertainment of signs and symptoms (signs and symptoms not completely captured in medical charts). Such biases may have affected the validity of these results.[51,52] Furthermore, the details of reporting across studies varied greatly with only one study collecting daily data on bleeding. Unstructured reporting (e.g., unprompted reporting to clinicians) or reporting over long periods (e.g., monthly) also may have introduced reporting error, and could have decreased the precision of the estimates. Caution is particularly warranted

in generalizing findings to losses occurring prior to care entry, which constituted the majority of losses.[4,53] Despite the biases inherent in these studies, the observed associations are biologically plausible.

Physiology of bleeding in relation to pregnancy outcomes

Bleeding may be a cause and/or consequence of pregnancy loss. Women who experience either a complete or incomplete abortion must also experience vaginal bleeding by clinical definition.[54] In these cases, bleeding is a consequence of a loss, as this bleeding occurs concurrently with the expulsion of the products of conception from the uterus. Not all women, however, experience bleeding prior to recognition of the pregnancy loss. This is the case in women experiencing a missed abortion.

Subchorionic hemorrhage, which is bleeding between the uterine wall and the chorion detected by ultrasonography, is often, though not always, associated with vaginal bleeding and has been described as a cause of pregnancy loss.[55] Mechanisms have been proposed to explain the consequence of pregnancy loss resulting from bleeding during pregnancy. Johns and colleagues[56] have suggested that bleeding early in pregnancy causes increased oxygenation of the embryonic environment, which interferes with embryonic and placental development resulting in pregnancy loss. Under this hypothesis, oxygenation beyond what is chemically required during early gestation results in the formation of reactive oxygen species (ROS) that interfere with trophoblastic cells during a period of rapid cell division that is particularly sensitive to insults from ROS. Subchorionic bleeding during pregnancy is believed to be one pathway by which the

oxygen-rich maternal blood supply prematurely perfuses the intervillous space.[56] Chronic inflammatory processes associated with subchorionic bleeding/hematoma may also lead to myometrial contractions and expulsion of the gestational sac.[56]

Physiology of nausea and vomiting in relation to pregnancy outcomes

Two hypotheses promote NVP as the cause of healthy pregnancies: the "maternal-embryo protection hypothesis" advanced by Hook,[57] Profet,[58] and Sherman and Flaxman[59] and the "growth generating hypothesis" proposed by Huxley.[60] Under the maternal-embryo protection hypothesis, NVP functions to reduce consumption of potentially harmful foods (e.g., plants with phytotoxins or meats contaminated with parasites or pathogens) during the period of organogenesis to prevent congenital malformations or pregnancy loss.[61] Indeed, women report aversions to meat, alcohol, and caffeine during early pregnancy with an increased preference for carbohydrate-rich foods.[61] Under the growth generating hypothesis, caloric energy restriction secondary to NVP in the first trimester stimulates placental growth, which is necessary to successfully maintain pregnancy. In response to reduced caloric intake, maternal levels of insulin and insulin growth factor-1 (IGF-1) fall. This in turn inhibits maternal anabolic processes and redistributes nutrient partitioning to favor placental development.[60]

An alternative hypothesis suggests that NVP is a consequence of an already well-developing pregnancy.[62,63] As low hCG levels can be associated with both failing pregnancy and an absence of NVP, NVP may merely be an indicator of the embryo quality or viability,[62] resulting in reverse causation. This theory attributing NVP to higher levels of maternal hCG is based on both

NVP and hCG peaking at around 12 to 14 weeks of gestation.[64] Furthermore, NVP is reported more commonly in pregnancies with elevated hCG levels, including multiple gestations, molar pregnancies, and pregnancies with female fetuses,[65] and hCG levels correlate with severity of NVP [66]. NVP may also serve as a proxy for progesterone levels.[67] High progesterone levels are necessary to maintain a successful pregnancy,[68] and higher progesterone levels are associated with NVP, potentially because of its effects on smooth muscle relaxation and consequent gastric dysrhythmia.[67]

NVP may also serve as a marker for length of gestation, which is itself associated with viability of the pregnancy, again resulting in reverse causation. NVP peaks late in the first-trimester when most pregnancy losses have already occurred. Thus, pregnancies ending in early losses have less time at risk for NVP and their time at risk occurs when NVP is less prevalent; however, pregnancies ending in live births have greater time at risk for NVP and this time at risk encompasses the period of high NVP prevalence. This differential time at risk for NVP may explain the association between absence of NVP and loss. If one considered the effect of NVP during the early first trimester when losses are most likely to occur and NVP is relatively less common across all pregnancies, one may see a different relationship between NVP and loss (possibly, a null association).

Limitations of existing literature on signs and symptoms of pregnancy loss

Cumulative incidence of hCG pregnancy loss (~ 25-33%)[4,10,11,14] is roughly double the cumulative incidence of clinically recognized pregnancy loss (~10-15%).[4,10,11,14] Thus, in

pregnancy cohort studies many of the pregnancy losses occurring early in gestation were either not captured at all or data on signs and symptoms were ascertained after the loss was recognized. Therefore, data from these pregnancy cohorts must be interpreted with caution, as the incidence of signs and symptoms likely did not include early losses in either the numerator (number of losses) or the denominator (number of pregnancies). Of note, I only included studies that attempted prospective ascertainment of signs and symptoms to limit recall bias i.e., studies involving data collection that preceded the *ascertainment* of the pregnancy loss. However, the day of loss was often unknown, and thus, data on signs and symptoms may have been collected after the loss of the pregnancy but prior to loss recognition.

As healthier pregnancies tend towards longer gestations than unhealthy pregnancies, pregnancy cohorts capture more healthy pregnancies and fewer unhealthy pregnancies than the underlying source population of all pregnancies, resulting in length-biased sampling. The pregnancies observed in typical pregnancy cohorts are less likely to end in a loss and possibly more likely to have signs and symptoms of pregnancy such as nausea and vomiting simply because of the gestational age at which signs and symptoms are ascertained. Results from these studies may not be applicable to very early pregnancy losses as the relationships between signs and symptoms and pregnancy loss may change across gestation. Furthermore, the reported associations may be affected by recall bias if data on signs and symptoms were collected after a loss.

CONCLUSIONS

Existing data provided some insights into the relationships between individual signs and symptoms and pregnancy loss among care-seeking populations with gestations that are well into

the first-trimester and beyond. These included the findings that vaginal bleeding was associated with an increased risk of pregnancy loss and that nausea and vomiting were associated with decreased risks of pregnancy loss. However, three notable data gaps exist. First, data are needed on early first trimester pregnancy losses, particularly those that would not normally reach clinical care but which comprise a large proportion of pregnancy losses. Second, data on multiple signs and symptoms captured simultaneously are needed to establish temporal patterns of signs and symptoms that may be concerning or reassuring for subsequent pregnancy loss and to allow empirical testing for time-varying effects of signs and symptoms on losses across gestation, e.g., are the relationships between signs and symptoms and loss consistent throughout gestation or do they vary by gestational age? Third, detailed data on gestational age at pregnancy discovery, loss ascertainment, loss to follow-up and birth are needed to employ survival analytic approaches to the signs and symptoms of pregnancy loss. To address these data gaps, preconception cohorts with detailed, prospectively collected data on multiple signs and symptoms and with uniform and accurate measures of gestational age are needed.

Table 2.1.A. Literature search terms in PubMed/MEDLINE

Applying the 'prospective studies', 'English', and 'Humans' MeSH term restrictions

abortion, spontaneous (MeSH Term) and nausea (Title/Abstract)

abortion, spontaneous (MeSH Term) and vomiting (Title/Abstract)

abortion, spontaneous (MeSH Term) and cramping (Title/Abstract)

abortion, spontaneous (MeSH Term) and bleeding (Title/Abstract)

abortion, spontaneous (MeSH Term) and (symptoms (Title/Abstract) or signs (Title/Abstract))

fetal death (MeSH Term) and nausea (Title/Abstract)

fetal death (MeSH Term) and vomiting (Title/Abstract)

fetal death (MeSH Term) and cramping (Title/Abstract)

fetal death (MeSH Term) and bleeding (Title/Abstract)

fetal death (MeSH Term) and (symptoms (Title/Abstract) or signs (Title/Abstract))

pregnancy loss (Title/Abstract) and nausea (Title/Abstract)

pregnancy loss (Title/Abstract) and vomiting (Title/Abstract)

pregnancy loss (Title/Abstract) and cramping (Title/Abstract)

pregnancy loss (Title/Abstract) and bleeding (Title/Abstract)

pregnancy loss (Title/Abstract) and (symptoms (Title/Abstract) or signs (Title/Abstract))

Applying the 'Humans' and 'English' MeSH term restrictions

miscarriage (Title/Abstract) and vaginal bleeding (Title/Abstract)

miscarriage (Title/Abstract) and nausea (Title/Abstract)

miscarriage (Title/Abstract) and symptoms (Title/Abstract)

pregnancy loss (Title/Abstract) and pregnancy symptoms (Title/Abstract)

Applying only the 'English' restriction

Pregnancy Complications[MeSH Major Topic] AND bleeding[Title/Abstract]

Pregnancy Complications[MeSH Major Topic] AND (vomiting[Title/Abstract] or nausea[Title/Abstract])

Pregnancy Complications[MeSH Major Topic] AND cramping[Title/Abstract]

Table 2.1.B Literature search terms in Embase

Applying the 'English', and 'Humans' limitations

spontaneous abortion and nausea

spontaneous abortion and vomiting

spontaneous abortion and cramping

spontaneous abortion and bleeding

spontaneous abortion and (symptoms or signs)

fetal death and nausea

fetal death and vomiting

fetal death and cramping

fetal death and bleeding

fetal death and (symptoms or signs)

pregnancy loss and nausea

pregnancy loss and vomiting

pregnancy loss and cramping

pregnancy loss and bleeding

pregnancy loss and (symptoms or signs)

miscarriage and vaginal bleeding

miscarriage and nausea

miscarriage and symptoms

pregnancy loss and pregnancy symptoms

Pregnancy Complications AND bleeding

Pregnancy Complications AND (vomiting or nausea)

Pregnancy Complications AND cramping

Table2. 2. Cumulative incidence of vaginal bleeding and associations with pregnancy loss

First author, year [ref #]	Sampling frame and study size (n)	Assessment of bleeding and pain	GA at assessment	Cumulative incidence of bleeding	Cumulative incidence of loss in women with bleeding	Cumulative incidence of loss in women without bleeding	Risk ratio and 95% CI
Care-seeking cohorts							
Peckham, 1970 [35]	Patients with pregnancies that ended (loss or delivery) in the Kaiser Permanente Child Health and Development Studies (n=6,223).	Self-report of vaginal bleeding noted in clinic charts; divided women into those with bleeding ≤6 days and ≥7 days from loss or delivery.	1st, 2nd, 3rd trimesters	19% of women had bleeding reported in clinic charts; 15% of women had onset of bleeding ≥7 days from end of pregnancy.	14% of women with bleeding whose pregnancies ended ≥7 days after onset of bleeding had a loss.	2% of women who did not have a record of bleeding experienced a loss.	RR: 8.6 (6.6, 11.2)
Everett, 1997 [34]	Women with a positive pregnancy test at a general practice in England who continued their pregnancies (n=550).	Self-report of vaginal bleeding recorded in practice notes or hospital discharge note.	1st, 2nd, 3rd trimesters	21% of women had record of bleeding <20 weeks GA.	56% of women with bleeding <20 weeks GA had a loss.	0.5% of women without bleeding had a loss.	RR: 120 (30, 484)

Table2. 2. Cumulative incidence of vaginal bleeding and associations with pregnancy loss (cont.)

First author, year [ref #]	Sampling frame and study size (n)	Assessment of bleeding and pain	GA at assess-ment	Cumulative incidence of bleeding	Cumulative incidence of loss in women with bleeding	Cumulative incidence of loss in women without bleeding	Risk ratio and 95% CI
Care-seeking cohorts (continued)							
Makrydimas et al., 2003 [33]	Women with a viable fetus on routine ultrasound scan at 6-10 at a university hospital in Greece (n=668).	Self-report of bleeding recorded in hospital notes.	1st, 2nd, 3rd trimesters	7% of women had bleeding recorded in hospital notes.	17% of women with bleeding had a loss.	7% of women without bleeding had a loss.	RR: 2.6 (1.3, 5.2)
Weiss et al., 2004 [36]	Women with a viable pregnancy on ultrasound enrolled at 10-14 weeks GA in a trial for trisomy 21 screening in an unselected obstetric population (n=16,506).	Self-report of no, light (spotting), heavy (similar to menses) bleeding in the 4 weeks prior to enrollment.	1st trimester	14% of women reported bleeding in 4 weeks prior to enrollment (13% light, 1% heavy).	1% of women with light bleeding and 2% of women with heavy bleeding had a loss <24 weeks GA.	0.4% of women with no bleeding in 4 weeks prior to enrollment had loss <24 weeks GA.	Overall RR: 2.8 (1.7, 4.4) Light vs no RR: 2.5 (1.5, 4.1) Heavy vs no RR: 4.9 (2.0, 12.2)

Table2. 2. Cumulative incidence of vaginal bleeding and associations with pregnancy loss (cont.)

First author, year [ref #]	Sampling frame and study size (n)	Assessment of bleeding and pain	GA at assess-ment	Cumulative incidence of bleeding	Cumulative incidence of loss in women with bleeding	Cumulative incidence of loss in women without bleeding	Risk ratio and 95% CI
Community-based cohorts							
Harville et al., 2003 [38]	Women in North Carolina enrolled in a preconception cohort and whose pregnancies lasted >6 weeks	Self-report of any vaginal bleeding and number of tampons or pads used; excluded bleeding associated with expulsion of fetus (not defined).	1st tri-mester	9% of women with pregnancies ≥6 weeks GA reported bleeding ≤8 weeks GA.	14% of women with bleeding ≤8 weeks GA had a loss.	9% of women without bleeding ≤8 weeks GA had a loss.	RR: 1.5 (0.4, 6.0)
Hasan et al., 2009 [37]	Women recruited in early pregnancy (<12 weeks GA) or prior to pregnancy in 3 Southern cities in the United States; enrolled after positive pregnancy test (n=4,510).	Self-report of all episodes of bleeding in 1st trimester including number of episodes, date of onset, duration, heaviness, color was reported at end of first trimester; excluded bleeding within 4 days of loss; self-report of pain as mild, moderate, or severe; allowed for reporting after loss.	1st tri-mester	27% of women reported 1st trimester bleeding; 8% of women with bleeding reported heavy (≥heaviest day of menses) episodes.	11% of women with any bleeding had a loss. 24% of women with heavy bleeding had a loss. 8% of women with heavy 1st trimester bleeding had 2nd trimester loss.	12% of women without 1st trimester bleeding had a loss. 1% of women without 1st trimester bleeding had a 2nd trimester loss.	Any vs no RR: 0.9 (0.8, 1.1) Heavy vs no RR: 2.0 (1.4, 3.0) Heavy /pain vs none RR: 3.1 (2.1, 4.5)

Table 2.3. Cumulative incidence of nausea and vomiting in pregnancy and associations with pregnancy loss

First author, year [ref #]	Sampling frame and n	Assessment of NVP	GA at NVP assessment	Cumulative incidence of NVP	Cumulative incidence of loss in women with NVP	Cumulative incidence of loss in women without	Risk ratio and 95% CI
Care-seeking cohorts							
Speert and Guttmacher, 1954 [42]	Private patients in NYC (n=256).	Self-report of NVP.	1st trimester	65% of women reported NVP.	5% of women with NVP had a 1st trimester loss.	26% of women without NVP had a 1st trimester loss.	NVP RR: 0.2 (0.1, 0.4)
Medalie 1957 [41]	Patients in a general practice in rural Israel (n=100).	Self-report of NVP (mild, moderate, severe based on daily frequency).	1st trimester	71% of women had NVP; 52% had moderate/ severe NVP.	0% of women with moderate/ severe NVP had a 1st trimester loss.	23% of women with mild/no NVP had a 1st trimester loss.	Cannot calculate
Brandes, 1967 [39]	Patients with singleton pregnancies receiving prenatal care in Kaiser Permanente and participating in a study (n=7,027).	Self-report of NVP.	1st trimester	73% of women had NVP.	5% of women with NVP experienced a loss.	9% of women without NVP experienced a loss.	NVP RR: 0.6 (0.5, 0.7)

Table 2.3. Cumulative incidence of nausea and vomiting in pregnancy and associations with pregnancy loss (cont.)

First author, year ref #	Sampling frame and n	Assessment of NVP	GA at NVP assess-ment	Cumulative incidence of NVP	Cumulative incidence of loss in women with NVP	Cumulative incidence of loss in women without NVP	Risk ratio and 95% CI
Care-seeking cohorts(continued)							
Kullander and Kallen, 1976 [47]	Pregnant women in Malmo, Sweden between 1963-65 with loss or birth without congenital anomalies (n=5,377)	Self-report of NVP by questionnaire.	1st, 2nd, 3rd tri-mester	72% of women reported NVP.	5% of women with NVP had a loss.	14% of women without NVP had a loss.	NVP RR: 0.4 (0.3, 0.4)
Klebanoff et al., 1985 [40]	Women registered in the National Collaborative Perinatal Project <14 weeks GA with ongoing pregnancy at 14 weeks GA (n=9,098).	Query of vomiting at each obstetric visit.	1st tri-mester	52% of women had vomiting by 16 weeks GA.	3% of women with vomiting had a loss.	5% of women without vomiting had a loss.	Vomiting RR: 0.6 (0.5, 0.8)
Tierson et al., 1986 [43]	Predominately white, upper-class women attending private practice in Albany, NY who had ongoing pregnancy at 12 weeks GA (n=414).	Self-report of NVP at interview at 12 weeks GA and then every 2 weeks until 20 weeks GA.	1st and 2nd tri-mester	89% of women reported NVP by 20 weeks GA; 56% reported vomiting.	7% of women with any NVP, 10% of women with nausea only, 5% of women with vomiting had a loss.	20% of women without NVP had a loss.	NVP RR: 0.5 (0.2, 0.9) Nausea only RR: 0.5 (0.2, 1.1) Vomiting RR: 0.3 (0.1, 0. 6)

Table 2.3. Cumulative incidence of nausea and vomiting in pregnancy and associations with pregnancy loss (cont.)

First author, year ref #	Sampling frame and n	Assessment of NVP	GA at NVP assess-ment	Cumulative incidence of NVP	Cumulative incidence of loss in women with NVP	Cumulative incidence of loss in women without NVP	Risk ratio and 95% CI
Care-seeking cohorts(continued)							
Weigel and Weigel, 1989 [45]	Women delivering babies at US hospital ≥21 weeks GA or miscarriage <21 weeks GA with >1 prenatal visit <21 weeks GA (n=903).	Self-report of NVP recorded in the medical chart.	1st and 2nd tri-mester	69% of women had NVP <21 weeks GA; 23% had nausea only; 46% had vomiting and nausea.	2% of women with NVP, 4% of women with nausea only, 1% of women with vomiting had loss.	7% of women without NVP recorded in medical charts had a loss.	NVP RR: 0.3 (0.1, 0.5) Nausea only RR: 0.5 (0.2, 1.2) Vomiting RR: 0.1 (0.1, 0.4)
Weigel et al., 2006 [44]	Women in 1st trimester of pregnancy receiving prenatal care at a public hospital in Quito, Ecuador (n=849).	Self-report of NVP: interviews in 1st and 2nd trimester: nausea only and nausea and vomiting.	1st and 2nd tri-mester	77% of women reported NVP by 20 weeks GA; 21% had nausea only; 56% had nausea and vomiting.	3% of women with NVP, 4% of women with nausea only, 2% of women with vomiting had a loss.	7% of women without NVP had a loss.	NVP RR: 0.4 (0.3, 0.9) Nausea only RR: 0.7 (0.3, 1. 6) Vomiting RR: 0.3 (0.2, 0.8)
Weng et al., 2008 [46]	Women with a positive pregnancy test at Kaiser Permanente in San Francisco (n=1,063).	Self-report of NVP prior to interview; allowed report after loss.	Early 1st tri-mester	78% of women reported NVP; 40% of women reported vomiting.	11% of women with NVP had a loss.	35% of women without NVP had a loss.	NVP RR: 0.3 (0.2, 0.4)

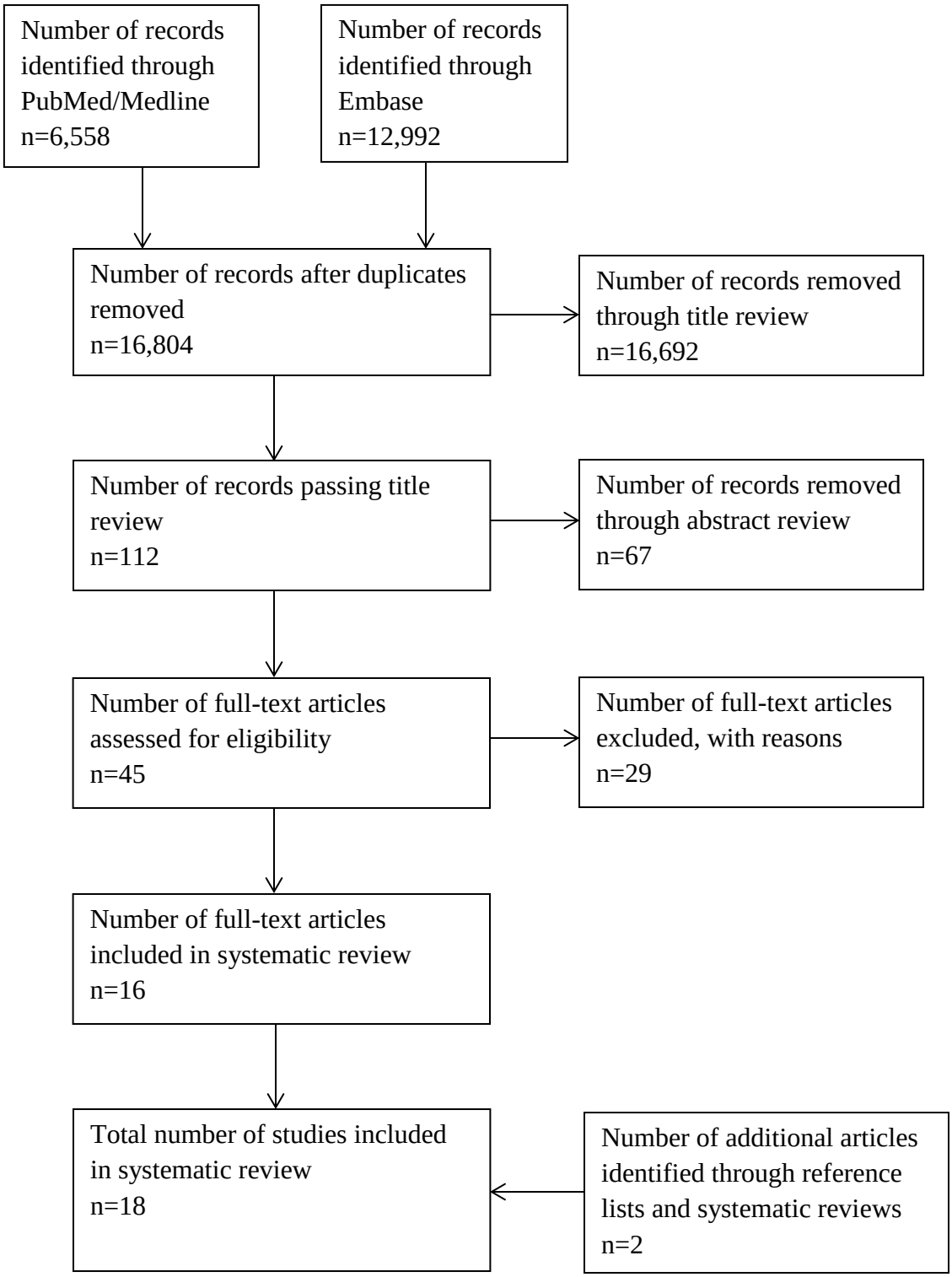
Table 2.3. Cumulative incidence of nausea and vomiting in pregnancy and associations with pregnancy loss (cont.)

First author, year ref #	Sampling frame and n	Assessment of NVP	GA at NVP assess-ment	Cumulative incidence of NVP	Cumulative incidence of loss in women with NVP	Cumulative incidence of loss in women without NVP	Risk ratio and 95% CI
Community-based cohorts							
Wen et al., 2001 [49]	Women in a preconception population-based cohort in Twin Cities, Minnesota with data on nausea (n=585).	Monthly self-report of nausea including duration in days; allowed for reporting after loss.	1st tri-mester	88% of women reported nausea in 1st trimester.	7% of women with nausea in the first trimester had a loss.	30% of women without nausea in the 1st trimester had a loss.	NVP RR: 0.2 (0.2, 0.4)
Chan et al., 2010 [48]	Women recruited in early pregnancy (<12 weeks GA) or prior to pregnancy in 3 Southern cities in the United States; enrolled after positive pregnancy test (n=2,407).	Self-report of NVP onset and cessation of reported at enrollment (<16 weeks GA) and at follow-up (20-25 weeks GA); allowed reports after loss.	1st and 2nd tri-mester	89% of women reported NVP in 1st or 2nd trimesters; 53% reported vomiting.	Not reported.	Not reported.	No NVP vs Vomiting Odds Ratio: 5.7 (4.0, 8.0) Nausea only vs Vomiting Odds Ratio: 2.4 (1.8, 3.3)

Table 2.4. Cumulative incidence of nausea/vomiting in the setting of vaginal bleeding and associations with pregnancy loss

First author, year [ref #]	Sampling frame, assessment of bleeding, and study size (n)	Assessment of bleeding and NVP	GA at assess-ment	Cumulative incidence of NVP among women with bleeding	Cumulative incidence of loss in women with bleeding and NVP	Cumulative incidence of loss in women with bleeding/ without NVP	Risk ratio and 95% CI
Care-seeking cohorts							
Medalie 1957 [41]	Patients in a general practice in rural Israel with threatened abortion (n=23).	Self-report of NVP; moderate NVP = extreme nausea all day or vomiting ≥2/day; severe NVP =vomiting almost every meal.	1st tri-mester	4% of women with bleeding reported moderate/ severe NVP.	0%; only 1 woman reported both severe/ moderate NVP and bleeding; not a loss.	50% of women with bleeding and with no/mild NVP had a loss.	Cannot calculate.
Kouk et al., 2013 [50]	Patients with bleeding 5-10 weeks GA presenting to hospital in Singapore were followed until 16 weeks gestation for loss (n=139).	Self-report of nausea prior to presentation for evaluation.	1st tri-mester	41% of women with bleeding reported history of nausea.	~38% of women with both bleeding and nausea had a loss.	~12% of women with bleeding but without nausea had a loss.	Hazard ratio: 0.3 (0.1, 0.6)

Figure 1. Flowchart for identification, exclusion, and inclusion of studies



CHAPTER 3: SIGNS AND SYMPTOMS ASSOCIATED WITH EARLY PREGNANCY LOSS: FINDINGS FROM A POPULATION-BASED PRECONCEPTION COHORT

ABSTRACT

The objective of this study was to assess the relationships between signs and symptoms of early pregnancy and pregnancy loss <20 weeks gestation using a population-based preconception cohort of 501 couples discontinuing contraception to try for pregnancy in 16 counties in Michigan and Texas, USA. Participants were followed daily until positive home pregnancy test or 12 months of trying without an hCG pregnancy; women who became pregnant recorded daily from 2 to 7 weeks post-conception their signs and symptoms, including vaginal bleeding (none, spotting, light, moderate, heavy), lower abdominal cramping, nausea, and vomiting, which were classified as any/none during the early pregnancy period. Individual signs and symptoms, their combinations, and their temporal patterning were evaluated in relation to pregnancy loss. Pregnancy losses were ascertained by a conversion to a negative home pregnancy test, clinical confirmation, or onset of menses, depending on gestational age at demise; time-to-loss was measured in days post-conception. Cumulative incidence functions and 95% confidence intervals were constructed for each sign or symptom, and hazard ratios and 95% confidence intervals for presence compared with absence of signs or symptoms were estimated using Cox proportional hazard models.

Ninety-five (28%) women experienced a loss. Women experienced lower abdominal cramping (85%), nausea (48%), vomiting (46%), and light/moderate/heavy vaginal bleeding (24%) during

early pregnancy. Ten percent of women experienced no vomiting, cramping, or bleeding, 36% experienced cramping only, 26% experienced cramping with vomiting, 8% experienced cramping with bleeding, and 15% experienced all 3 symptoms. Cumulative incidence of pregnancy loss varied by individual signs and symptoms: 19% for vomiting, 27% for cramping, 35% for nausea only, 50% for bleeding. Cumulative incidence of loss also varied by combinations of signs and symptoms: 10% for cramping with vomiting, 23% for cramping alone, 34% for no symptoms, 36% for all 3 symptoms, and 81% for bleeding with cramping. Vaginal bleeding was associated with increased incidence of early pregnancy loss (HR 3.62, 95% CI 2.29, 5.74), with more severe bleeding and bleeding with lower abdominal cramping associated with greater incidence of loss (HR 5.03, 95% CI 2.07, 12.20); conversely, vomiting was associated with a decreased incidence of early pregnancy loss (HR 0.51, 95% CI 0.30 to 0.86), while nausea alone was not. In the setting of vaginal bleeding with lower abdominal cramping, vomiting reduced the incidence of pregnancy loss (hazard ratio 0.24, 95% confidence interval 0.11 to 0.56).

By using sensitive home pregnancy tests, I was able to document and characterize the cumulative incidence of the earliest pregnancy losses, which constituted the majority of losses. The use of daily, prospective capture of signs and symptoms relative to ascertainment of pregnancy loss avoided potential biases associated with reporting after rather than before a loss, which could potentially distort the relationship between signs and symptoms and pregnancy loss. The findings of this study suggest that it may be useful to develop prognostic models for pregnancy loss based on signs and symptoms; such models may need to incorporate potentially time-varying effects of signs and symptoms on pregnancy loss which will be explored in the Chapter 4.

INTRODUCTION

Pregnancy loss is common, affecting approximately one-third of pregnancies.[4,14] However, among spontaneous conceptions, where the endpoints of fertilization and implantation are not readily visualized, loss is often unobserved. This problem contributes to our limited understanding of the earliest stages of pregnancy and human development. In fact, the natural history of pregnancy loss, including temporal ordering of signs and symptoms, has yet to be fully described. As reviewed in Chapter 2, there is a dearth of studies with prospectively collected information on the signs and symptoms of pregnancy loss. Valid data on signs and symptoms that portend pregnancy loss would be useful for women and clinicians to prompt medical care and evaluation for women experiencing concerning signs and symptoms. However, valid data can only be obtained from preconception studies, which is the only study design that facilitates the prospective ascertainment of the earliest signs and symptoms prior to any subsequent loss.

Despite this, only two reported studies with preconception enrollment were identified in the literature. One study evaluated daily vaginal bleeding[38] and non-specific pregnancy symptoms,[69] separately, in relation to pregnancy loss but was limited by the small number of losses (n=62).[4] Another study evaluated monthly reports of nausea in relation to loss but reporting often occurred after the loss.[49] As signs and symptoms do not occur in isolation, studies describing multiple signs and symptoms simultaneously in relation to loss are needed to delineate the natural history of pregnancy loss. I identified three such studies in Chapter 2, all conducted among women seeking clinical care. Two were pregnancy cohort studies conducted in the 1950s,[41,42] while the other more recent study only recruited women presenting for evaluation of

bleeding during pregnancy.[50] Thus, there is a distinct gap in the literature regarding the symptomatology associated with early pregnancy and early pregnancy loss for contemporary cohorts of women who are now able to recognize pregnancy earlier than previous birth cohorts; in the era of home pregnancy testing, many women may detect their pregnancies, and even early losses, before presenting for clinical care.[70] Data are needed from non-clinical cohorts that can prospectively and continuously ascertain multiple signs and symptoms early in pregnancy and prior to loss ascertainment.

I, therefore, undertook this study to examine the relationship between multiple signs and symptoms—vaginal bleeding, lower abdominal cramping, nausea, and vomiting—and pregnancy loss at less than 20 weeks gestation in a population-based preconception cohort of women. My objective was to describe the symptomatology of early pregnancy loss using a unique data set of pregnancies ascertained early using home pregnancy tests with daily prospective collection of signs and symptoms prior to pregnancy loss ascertainment. As the majority of early pregnancy losses occur prior to clinical care,[4,49] this study cohort offered a unique opportunity to delineate the signs and symptoms occurring in the first few days of pregnancy before gestational demise.

METHODS

Study population

Details of the recruitment strategy for the Longitudinal Investigation of Fertility and the Environment (LIFE) Study have been described elsewhere.[71] Briefly, the LIFE Study was a

population-based, prospective, preconception cohort study of couples attempting pregnancy conducted from 2005-2009 in 16 counties in Michigan and Texas, USA. Couples who were planning to discontinue contraception to become pregnant and those who had been attempting pregnancy for ≤ 2 months were screened for study eligibility. Eligible couples were those who were married or in a committed relationship, in which both partners were able to communicate in English or Spanish, the male partner was aged 18 years or older, and the female partner was aged 18-40 years, had a usual cycle length of 21-42 days, and no hormonal birth control injections in the past year. Couples who had clinically diagnosed infertility and those in which at least one partner was sterilized were excluded. All women had a urine-based pregnancy test administered at the baseline home interview to ensure they were not already pregnant and still at risk for pregnancy upon study entry.

Study measures

Maternal characteristics

At baseline interview, women reported their age, race/ethnicity, education, income, employment status, current smoking status, and reproductive history (past pregnancies, deliveries, and pregnancy losses). Study personnel measured the women's height and weight using standardized protocols in order to calculate body mass index (BMI).[72]

Ascertainment of conception

In the absence of visualization of either ovulation or conception, we used proxy markers of ovulation and conception. At study entry, women were instructed to use the urine-based digital

ClearBlue ™ Fertility Monitor consistent with the manufacturer's guidance. The monitor records the ratios of estrone-3-glucuronide and luteinizing hormone and stores summary data for up to 6 months. Study personnel downloaded the data every 45 days per the terms of a Disclosure Agreement with the manufacturer and *Eunice Kennedy Shriver* National Institute of Child Health and Human Development. The ClearBlue™ Fertility Monitor has been demonstrated to provide an accurate measure of ovulation compared with the gold standard, i.e., ultrasound visualization of ovarian follicles and ovulation.[73] Day of ovulation was approximated by the peak day of luteinizing hormone as indicated by the fertility monitor. If two days of peak luteinizing hormone were indicated, the latter day was taken as the day of ovulation.[73] For 59 women (17%) who did not have fertility monitor data available for the pregnancy cycle, data from the fertility monitor were available for other menstrual cycles within the same woman that did not end in pregnancy. The average day of ovulation from the prior cycles was imputed as the day of ovulation for the pregnancy cycle. For an additional 16 women (5%) with no fertility monitor information for any cycles in the study, ovulation was assumed to occur 14 days prior to the positive pregnancy test, consistent with the relatively more stable length of the secretory phase of the menstrual cycle compared with the proliferative phase.[74] As previously suggested,[75] the day of conception was approximated by the day of ovulation in keeping with the short viability of the ovum following ovulation.[76]

Ascertainment of pregnancy

Pregnancy was established by the urine-based home pregnancy test. All women were provided with ClearBlue™ Digital Pregnancy Test (Inverness Medical Innovations, Waltham, MA) kits and multiple urine test sticks for each cycle. Women were instructed to test on the day of

expected menses. If the test was positive, they were instructed to test again one week after the first positive pregnancy test. If the pregnancy test on the day of expected menses was negative, women were instructed to test again in one week or if bleeding began. These instructions are consistent with manufacturer's guidance. The test has an advertised hCG sensitivity of 25 mIU/mL, though independent testing has shown that it can detect even lower concentrations of pure hCG and hyperglycosylated hCG, the predominant forms of hCG in early pregnancy.[73] The digital readout of 'pregnant' and 'not pregnant' removed subjectivity in interpreting the results associated with symbols or colored lines. Women recorded daily whether they took a pregnancy test and the result of the test. One positive urine pregnancy test denoted an hCG pregnancy.

Ascertainment of signs and symptoms

Signs and symptoms of pregnancy and associated loss were recorded daily for five weeks beginning on the day after the positive pregnancy test (~2 to 7 weeks post-conception). If women experienced a pregnancy loss during that interval, only information on signs and symptoms occurring before the day of event were used. Vaginal bleeding was recorded as none, spotting, light, moderate, or heavy using standardized pictographs.[77] Lower belly cramping was recorded as present or absent. Nausea and vomiting were recorded as none, nausea only, vomiting only, or both nausea and vomiting. Women had the option of completing journals online daily or on hardcopy. If the former, women could not edit previously submitted data unless they notified the data coordinating center they made a mistake. If the latter, women were instructed to not backfill any days they missed; they were instructed to leave those days missing. The hardcopy journals queried daily for one week before the postcard with information was returned. Research

assistants monitored the web based data collection system to ensure cards were returned in a timely manner.

Ascertainment of pregnancy loss

Pregnancy loss was defined as conversion of a positive pregnancy test to a negative pregnancy test, clinical confirmation, or onset of vaginal bleeding consistent with expulsion of the products of conception.[78] More details are provided in Appendix A. Early pregnancy loss was defined as a loss of an hCG pregnancy <20 weeks gestation. Ectopic pregnancies and pregnancy losses occurring ≥20 weeks gestational age were excluded.

Statistical analysis

Descriptive analyses

Several descriptive analyses were undertaken to understand the data. Given the multiple methods by which women could ascertain their losses depending upon gestational age, I assessed potential differences in day of positive pregnancy test, day of pregnancy loss ascertainment, and maternal characteristics by loss ascertainment method. I also examined the maternal characteristics of women included in the analytic cohort.

Multiple imputation of signs and symptoms

Despite the intensity of daily collection of signs and symptoms, the daily journal data were mostly complete. Seventy-six percent of women were missing less than 30% of daily bleeding

data, and fifty-nine percent of women were missing less than 30% of daily cramping, nausea, and vomiting data. Any missing daily data on signs and symptoms were imputed using the multiple imputation 'mice' package in the R software.[79] One hundred imputed data sets were generated. For each sign or symptom, all available data were used for the imputation, including maternal characteristics, other days of information on the sign or symptom, and other signs or symptoms. More detail is provided in Appendix B. Any imputed data occurring on or after the day of pregnancy loss or loss to follow-up were set to missing.

Cumulative incidence of signs and symptoms

Cumulative incidence functions (CIF) of pregnancy loss across gestation were constructed based on the imputed data.[80] CIF of loss by the presence and absence of the individual signs and symptoms, their severity, combinations, and patterns were constructed over time anchored to post-conception gestational age. As our definition of pregnancy loss only included losses <20 weeks gestation, any women with births or loss to follow-up after 20 weeks were censored at 140 days post-last menstrual period (LMP) or 125 days post-conception (assuming conception occurred 15 days post-LMP, which is 14 days prior to median positive pregnancy test at cycle day 29). Rubin's rules were used to combine CIF estimates and 95% confidence intervals (CI) across imputations. The log-log transformation was applied to 95% CI to ensure the resulting 95% CI of the loss probabilities were in the interval [0,1].

Signs and symptoms, combinations, and patterns of losses

The following categories were created for individual signs and symptoms and their severity: any cramping versus none; any bleeding (spotting, light, moderate, heavy bleeding) versus none; any light, moderate, heavy bleeding versus none/spotting only; any moderate/heavy bleeding versus none/spotting/light bleeding; any nausea and/or vomiting versus no nausea and/or vomiting; any vomiting (with or without nausea) versus no vomiting; and any vomiting (with or without nausea) versus nausea alone. 'Any' refers to the presence of the sign or symptom on one or more days during the early pregnancy period.

CIF were also constructed for combinations of three signs and symptoms: bleeding, vomiting, and cramping. Combinations that were considered positive for bleeding included women with one or more days of light, moderate, or heavy bleeding (women with spotting only were considered negative for bleeding). Combinations that were considered positive for vomiting included women with one or more days of vomiting (with or without nausea); women with nausea only were considered negative for vomiting. I fully explored all combinations of signs and symptoms and found that five combinations were sufficient for analysis: no bleeding/ cramping/vomiting, cramping alone, cramping with vomiting, cramping with bleeding, cramping with vomiting and bleeding. All other combinations were too sparse for stable estimates (<5% of imputations). Several reference groups were specified in individual models to better understand the relative hazards of combinations of symptoms relative to no symptoms, the most common symptom cramping only), and the combination of symptoms associated with highest risk of loss (cramping with bleeding).

I also examined the temporal order of the three signs and symptoms in relation to one another. The five combinations of the three signs and symptoms listed above yielded over two-dozen possible temporal patterns. I only analyzed patterns with sufficient sizes to allow for stable estimates (≥5% of imputations). These patterns were no bleeding/cramping/vomiting, cramping only, cramping followed by vomiting without bleeding, cramping followed by bleeding without vomiting, cramping followed by vomiting followed by bleeding, cramping following by bleeding followed by vomiting. Two reference groups were specified in individual models to better understand the relative hazards of combinations of symptoms relative to no symptoms and the pattern of symptoms associated with highest risk of loss (cramping followed by bleeding without vomiting).

Regression modeling of signs and symptoms and pregnancy loss

Cox proportional hazards models were used to estimate hazards ratios (HR) and 95% confidence intervals (CI) for individual signs and symptoms, combinations, and temporal patterns in relation to pregnancy loss. We examined all maternal characteristics for evidence of confounding; that is, characteristics associated with each sign or symptom and associated with loss among women without each sign or symptom. None of the characteristics met these criteria. We did not conduct any subgroup analyses as our objective was to describe the natural history of signs and symptoms of pregnancy loss among the entire population of women with an hCG pregnancy in this preconception cohort. As with the estimation of CIFs, post-conception gestational age was used as the anchor for survival time with censoring at 125 days for women who gave birth or were lost to follow-up ≥20 weeks gestation. Estimates were combined across imputations using

Rubin's rules implemented with PROC MIANALYZE in SAS version 9.4. I tested the proportionality assumption of the Cox proportional hazard model using a time-dependent covariate for the individual signs and symptoms, combinations, and patterns. Only moderate/heavy bleeding was time-dependent. On graphically assessing the non-proportionality by comparing the CIF graphs for time-to-loss, I did not detect extreme deviation from the proportionality assumption. As the Cox proportional hazard model is reasonably robust to the proportionality assumption,[81] in this Chapter I present the results for the fixed covariate.

RESULTS

Study sample

Of the 501 women enrolled, 347 became pregnant including 3 twin pregnancies. 341 remained in the study population after excluding 3 ineligible losses (Figure 3.1). Ninety-five (28%) women met the definition of having a pregnancy loss <20 weeks, 203 (60%) pregnancies ended in live birth, 24 (7%) in loss to follow-up before 20 weeks, and 19 (6%) in loss to follow-up after 20 weeks gestation.

The characteristics of 341 women are presented in Table 3.1. Almost half the women were aged thirty years or older (46%), the majority were non-Hispanic white (84%), had attended college (96%), had annual household incomes ≥$50,000 (87%), were employed (80%), were overweight or obese (50%), were recruited from Texas (81%), and did not smoke at study entry (93%). Thirty-nine percent of women had not been pregnant previously. Of 209 women with ≥1

previous pregnancies, 87% had ≥1 prior deliveries and 33% had ≥1 prior pregnancy losses (categories not mutually exclusive as women could have more than one prior pregnancy).

Median cycle day of first positive pregnancy test was day 29; this was the same for women with and without pregnancy loss (Appendix C). Among 95 women with losses, there were no differences in maternal characteristics of age, race/ethnicity, education, income, employment status, current smoking status, BMI, or reproductive history by the method of loss ascertainment (Appendix D). As expected, day of loss ascertainment differed by ascertainment method: losses at the earliest gestational ages were ascertained by bleeding pattern and negative pregnancy test, while losses at later gestational ages were ascertained by inaudible heartbeat and ultrasound confirmed fetal demise.

Cumulative incidence of signs and symptoms and relation to loss

A total of 335 (98%) women had one or more days of information on signs and symptoms between the positive pregnancy test and pregnancy outcome and were included in modeling. Of these 335 women, only 2% reported complete absence of any signs or symptoms during early pregnancy. Any nausea and/or vomiting was the most common symptom (94%); cumulative incidence of vomiting was 46% (Table 3.2). Cramping was also common affecting 85% of women. Cumulative incidence of any bleeding was 43%; cumulative incidence of bleeding exclusive of spotting was 24%. Ninety-nine percent of women had combinations of signs and symptoms that fell into the combinations modeled (Table 3.3). Eighty-seven percent of women had patterns that fell into the six patterns modeled; cramping only (36%), cramping followed by

vomiting without bleeding (24%), and no cramping/vomiting/bleeding (no symptoms) (10%) were more common than the three patterns with bleeding (5-6% each, Table 3.4).

Cumulative incidence functions

The overall CIF of loss for the sample is presented in Figure 3.2. The rate increased sharply from two (2%) to three weeks (10%) post-conception, and continued to rise until 10 weeks post-conception (27%) before plateauing at 14 weeks (28%). The expectation for the incidence of pregnancy loss in the absence of any additional information on signs and symptoms was 28% and could be regarded as a baseline cumulative incidence of early pregnancy loss <20 weeks gestation.

Figure 3.3 shows the CIF of pregnancy loss by presence or absence of any cramping; the incidence of loss was similar among women with and without cramping (~30%). Figure 3.4.A shows the CIF of loss for women with any versus no bleeding; a higher incidence of loss was observed among women with (~40%) than without bleeding (~20%). Figures 3.4.B and 3.4.C show the CIF of loss by severity of bleeding; incidence of loss exceeded 50% among women with more severe bleeding. The CIFs of loss by any and no nausea/vomiting are shown in Figure 3.5.A with similar incidence of loss among women with and without nausea/vomiting (~30%). Figures 3.5.B and 3.5.C show the CIF of loss by intensity of nausea/vomiting: none, nausea only, and any vomiting. Women with any vomiting had lower incidence of loss (~20%) than women with nausea only (~35%) and also those without any nausea or vomiting (~30%).

Figure 3.6 shows the CIF for the combinations of signs and symptoms. For women experiencing all three symptoms, the cumulative incidence of loss (~35%) was similar to the cumulative incidence of loss among women experiencing none of the three symptoms (~35%), which was only slightly greater than the baseline incidence of loss for the study population (~30%). However, when examined individually or in dual combinations, women with cramping only (~25%) and cramping with vomiting (~10%) had lower incidence of loss than comparison women. Women with bleeding and cramping had a markedly higher incidence of loss (~80%).

Figure 3.7 shows the CIF of loss for various patterns of signs and symptoms. Similar to Figure 3.6, the highest incidence of loss was observed for cramping with bleeding without vomiting (~80%) and the lowest incidence was for cramping with vomiting without bleeding (~10%). Cramping followed by bleeding followed by vomiting, and cramping followed by vomiting followed by bleeding had similar incidences of loss (~40%).

Cox proportional hazards models

Results of Cox proportional hazards models with individual signs and symptoms are presented in Table 3.5. Vaginal bleeding was associated with an increased incidence of pregnancy loss while vomiting was associated with a decreased incidence of pregnancy loss. Specifically, any versus no bleeding was associated with a higher incidence of loss (HR 2.65, 95% CI: 1.70 to 4.14). Similarly, light, moderate, or heavy bleeding versus none or spotting only (HR 3.62, 95% CI:

2.29 to 5.74) and moderate or heavy bleeding versus none, spotting, or light bleeding (HR 4.22, 95% CI: 2.53 to 6.69) were associated with an increased incidence of loss. Compared with no nausea and/or vomiting, nausea alone was not associated with pregnancy loss. However, any vomiting was inversely associated with loss compared with none (HR 0.51, 95% CI: 0.30 to 0.86) or compared with nausea alone (HR 0.50, 95% CI: 0.29 to 0.85). Presence compared with absence of cramping was not associated with loss.

Results of the Cox proportional hazards models with combinations of signs and symptoms are presented in Table 3.6 and show that signs and symptoms co-occurring with cramping were associated with variations in the incidence of pregnancy loss. Specifically, compared with no symptoms, cramping with vomiting was inversely associated with loss (HR 0.27, 95% CI: 0.10 to 0.78). Cramping with bleeding was positively associated with loss compared with no symptoms (HR 5.03, 95% CI: 2.07 to 12.20) or compared with cramping only (HR 7.26, 95% CI: 3.52 to 14.98). Cramping only, cramping with vomiting, and cramping with vomiting and bleeding were associated with lower incidences of pregnancy loss relative to cramping with bleeding.

Results of Cox proportional hazards models with various patterns of signs and symptoms are presented in Table 3.7 and reflect similar relationships to those seen with combinations of signs and symptoms. Compared with no symptoms, cramping followed by vomiting without subsequent bleeding was inversely associated with loss (HR 0.28, 95% CI: 0.10 to 0.82), while no association was observed for cramping followed by vomiting followed by bleeding.

Compared with no symptoms, cramping followed by bleeding without subsequent vomiting was positively associated with loss (HR 5.01, 95% CI: 1.84 to 13.67). Compared with cramping with bleeding without subsequent vomiting, cramping followed by bleeding followed by vomiting was inversely associated with loss (HR: 0.29, 95%CI: 0.09 to 0.96), as were cramping followed by vomiting followed by bleeding, cramping followed by vomiting without subsequent bleeding, and cramping only.

DISCUSSION

In this population-based preconception study of women who ascertained their pregnancy status using sensitive home pregnancy tests, multiple signs and symptoms were often reported in early pregnancy (approximately 2-7 weeks post-conception) and differed by pregnancy outcome. Lower abdominal cramping appeared to be the norm rather than the exception during early pregnancy, and cramping was not associated with pregnancy loss *per se*. Vaginal bleeding was associated with a higher incidence of pregnancy loss, and severe bleeding was associated with higher loss rates. Vomiting was associated with lower incidence of pregnancy loss, though nausea alone was not. Compared with cramping alone, cramping accompanied by bleeding was associated with the highest incidence of pregnancy loss; in contrast, cramping with vomiting was associated with the lowest incidence of pregnancy loss. For women with cramping followed by bleeding, the incidence of pregnancy loss was lower if vomiting occurred subsequently than if vomiting did not occur. For women with no cramping/vomiting/bleeding, the incidence of loss was only slightly greater than the baseline incidence of loss for the study population.

A notable strength of this work was that it utilized data from the largest prospective preconception cohort of couples who were followed daily[82] from enrollment through 7 weeks post-conception irrespective of pregnancy outcome. Second, highly sensitive home pregnancy tests were used to ascertain pregnancy status. Urine-based home pregnancy testing was less burdensome on participants than serial urine collection, was less costly, captured more pregnancies than waiting for clinical confirmation, and provided real-time feedback to couples. Third, offering women multiple methods by which to record a loss minimized the under-ascertainment of losses across all gestational ages. Importantly, all losses were recognized by the woman; none were 'silent' losses. I was therefore able to document and characterize the cumulative incidence of the earliest pregnancy losses, which constitute the majority of losses. Fourth, the study design allowed for the daily, prospective capture of signs and symptoms relative to ascertainment of pregnancy loss. This minimized potential biases associated with reporting after rather than before a loss, which could potentially distort the relationships between signs and symptoms and loss. While one cannot be certain that hardcopy journals were completed each day, I do not believe reporting differences varied by pregnancy outcome. Finally, I had a close proxy for the day of conception using fertility monitor data to ascertain the day of ovulation. Thus, I was able to use post-conception gestational age in survival analyses, which was a more precise measure of gestational duration than menstrual-based gestational age.

Study limitations included few losses beyond 14 weeks gestation because of the relatively small study size. Thus, the precision of the findings related to losses occurring after the first trimester was limited. Second, by study design, information on signs and symptoms was only collected daily from 2-7 weeks post-conception gestation consistent with the embryonic period of

development. Third, there were relatively small numbers within each pattern of signs and symptoms given the large numbers of possible patterns. Fourth, while women were instructed to test for pregnancy on the day of expected menses, the pregnancy test can detect pregnancies as soon as eight days after the luteinizing hormone surge.[83] There may be some biological variation in timing of positive pregnancy test relative to ovulation; however, measurement error of the proxy day of conception is unlikely to create substantial bias in our results given the small numbers of women impacted in our sample. Though small (7%), there was loss to follow-up prior to 20 weeks gestation in the study; this was addressed using a survival analytic censoring approach and available data was included in the study. Finally, there is some missing data on signs and symptoms. I did not find any statistically significant differences in the amount of missing information by the maternal characteristics that were used to inform the multiple imputation models. This provides some reassurance that the data are missing-at-random, which is an assumption challenging to verify in practice. Including several variables in the imputation model, as I did, also makes the missing-at-random assumption more plausible.[84] Furthermore, I generated 100 imputed dataset consistent with the amount of missing data.[85]

Given the amount of daily data available, I carefully considered which days of signs and symptoms to include in the analysis. I chose to include all of the days prior to the day of loss ascertainment for two reasons. First, understanding of the causes of pregnancy loss and the biologic mechanisms by which a pregnancy is spontaneously terminated is still very much unknown. Thus, one cannot say with any certainty that signs or symptoms occurring prior to a certain period before loss ascertainment are causes of loss while signs or symptoms occurring within a certain period of loss ascertainment are consequences of loss. Another serious concern

with excluding data on symptoms is that it would differentially impact early losses and bias the resulting estimates. Thus, I decided to include all data on signs and symptoms up until the day of loss ascertainment, acknowledging that day of loss ascertainment is an imperfect proxy for day of loss.

I did not categorize loss by gestational age in light of no uniformly agreed upon approach, particularly for non-clinical populations for whom most pregnancies and losses are observed only by the woman, and in the absence of a clear understanding of the etiology of pregnancy loss across gestation. The analyses presented here address the average impacts of signs and symptoms in early pregnancy on subsequent pregnancy loss irrespective of timing.

Few prior studies were able to evaluate multiple signs and symptoms in relation to pregnancy loss, and none explicitly examined lower abdominal cramping. Two pregnancy cohort studies in the 1950s[41,42] and a more recent study on threatened abortion[50] reported that in the setting of bleeding, nausea and/or vomiting was protective against pregnancy loss. Results presented in this Chapter were similar; however, in this study only vomiting was protective against loss in the setting of bleeding with cramping, while nausea was not.

Another pregnancy cohort study reported that the risk of pregnancy loss was greatest in women with heavy bleeding and pain.[37] I also found that the combination of bleeding with cramping was associated with the highest incidence of loss when it occurred without vomiting. A new finding

in this study was the high prevalence of lower abdominal cramping and the observation that cramping was not associated with loss unless accompanied by other signs or symptoms. Cramping accompanied by bleeding was associated with increased risk of loss; conversely, the risk of loss was decreased when cramping was accompanied by vomiting. This may suggest that the documentation of lower belly cramping in the daily journals captured a symptom that may have heterogeneous causes. Cramping that accompanies bleeding may reflect menstrual-type cramps that would be associated with the expulsion of the products of conception.[78] Uterine cramping associated with vomiting may be a different entity since uterine contractility is associated with higher estrogen levels, which are associated with a healthy and developing gestation.[86] Future epidemiologic studies may be able to ascertain the severity and typology of cramping in early pregnancy in more detail and more completely describe its relation to pregnancy loss.

Evaluating the temporal patterns of signs and symptoms in relation to loss was also novel. For women experiencing cramping and bleeding, subsequent vomiting was a significant prognostic indicator; women without subsequent vomiting were at increased risk of loss while women with subsequent vomiting were not. Collectively, these findings underscored the need to evaluate multiple signs and symptoms simultaneously, reflecting the co-occurrence of these symptoms for many women. For example, in this analysis, 85% of women had one or more symptoms; 40% of these women had two symptoms and 18% had all three symptoms.

Given the high frequency of lower abdominal cramping in early pregnancy and the lack of an association with pregnancy loss absent other co-occurring symptoms, it may be important to distinguish between different types of cramping, if such distinctions exist and can be made. In this study, the daily journals simply inquired about lower belly cramping and did not further qualify such as by severity or typology (e.g., menstrual-like cramps). While uterine quiescence is required for successful implantation of the embryo,[87] in the weeks following implantation uterine contractility may be associated with a healthy pregnancy. Potentially, this may be due to the effects of estrogen, which is associated with uterine contractility[87] and with ongoing pregnancy.[86] Cramping with bleeding likely reflects the expulsion of products of conception.[78] However, it is possible that bleeding is the cause, rather than the consequence, of pregnancy loss. Subchorionic hemorrhage, often associated with vaginal bleeding, may cause oxygen-rich blood to invade the intervillous space prematurely, interfering with trophoblast development,[56] or causing chronic inflammation, inducing myometrial contractions and expulsion of the gestational sac.[56]

In contrast to vaginal bleeding, vomiting in early pregnancy appeared to be protective against pregnancy loss. This is consistent with the hypothesis that caloric restriction consequent to vomiting in early pregnancy causes maternal levels of insulin and insulin growth factor-1 (IGF-1) to fall.[60] Maternal anabolic processes are thereby inhibited and nutrient partitioning favors placental development. Nausea may not cause caloric restriction to the same extent as vomiting, and the cascade described above would not be initiated. Alternatively, vomiting may serve as a proxy for high progesterone levels, which are necessary to maintain a successful pregnancy,[68] and are also associated with NVP, potentially through its effects on smooth muscle relaxation and consequent gastric dysrhythmia.[67] Visualized endpoints, such as embryo quality among

embryos being transplanted in assisted reproductive technology procedures, may be helpful in trying to disentangle some of these relationships between occurrence of signs and symptoms and pregnancy loss.

CONCLUSIONS

Though common in early pregnancy, lower abdominal cramping was not associated with pregnancy loss absent other signs and symptoms. Vomiting and nausea were also common in early pregnancy, and vomiting, but not nausea, was associated with a lower incidence of loss. While bleeding was less common in early pregnancy, it was associated with a higher incidence of loss, particularly if accompanied by lower abdominal cramping, though if vomiting subsequently followed bleeding the incidence of loss was less than if vomiting remained absent. The findings of this study suggest that it may be useful to develop prognostic models for pregnancy loss based on signs and symptoms; such models may need to incorporate potentially time-varying effects of signs and symptoms on pregnancy loss, which will be explored in the Chapter 4. More complete knowledge of the physiologic response of the body to early pregnancy will also enhance our understanding of the causes of each sign and symptom and its relation to pregnancy loss.

Table 3.1. Characteristics of women included in the study population (n=341)

	n (%) [a]
Age	
18-24 years old	25 (7)
25-29 years old	158 (46)
30-34 years old	114 (33)
35-40 years old	44 (13)
Race/Ethnicity	
Non-Hispanic White	283 (84)
Non-Hispanic Black	6 (2)
Hispanic	29 (9)
Other	20 (6)
Education	
High school or less	15 (4)
Some college or more	322 (96)
Income	
<$50,000	44 (13)
$50,000-99,999	161 (48)
$100,000+	127 (38)
Employed	
No	68 (20)
Yes	273 (80)
Body mass index	
<18.5	5 (1)
18.5-24.9	164 (48)
25.0-29.9	88 (26)
30.0+	83 (24)
Site	
Michigan	65 (19)
Texas	276 (81)

Table 3.1. Characteristics of women included in the study population (n=341) (cont.)

≥1 Prior pregnancy	
No prior pregnancy	132 (39)
Prior pregnancy	209 (61)
≥1Prior delivery (among those with ≥1 prior pregnancy)	
No prior delivery	27 (13)
Prior delivery	179 (87)
≥1 Prior loss (among those with ≥1 prior pregnancy)	
No prior loss	139 (67)
Prior loss	68 (33)
Current smoker	
No	318 (93)
Yes	23 (7)
	Median (IQR)
Cycle day of positive pregnancy test	29 (27, 32)

[a] May not add to total due to missing data

Table 3.2. Cumulative incidence of individual signs and symptoms and cumulative incidence of pregnancy loss by individual signs and symptoms

	Cumulative incidence of sign or symptom, %	Cumulative incidence of loss, %
Any Bleeding		
No	57	18
Yes	43	40
Bleeding Severity		
None/Spotting only	76	20
Light/moderate/heavy bleeding	24	52
Bleeding Severity		
None/Spotting/light bleeding	83	21
Moderate/heavy bleeding	17	57
Any Cramping		
No	15	31
Yes	85	27
Any Nausea and/or Vomiting		
No	6	30
Yes	94	27
Nausea and/or Vomiting Severity		
No nausea or vomiting	6	30
Nausea only	48	35
Any vomiting, with or without nausea	46	19
Any Vomiting		
No	54	34
Yes	46	19

a Does not add to 100% as some combinations too small for stable estimates and not included in modeling

Table 3.3. Cumulative incidence of combinations of signs and symptoms and cumulative incidence of pregnancy loss by combinations of signs and symptoms

	Cumulative incidence of sign or symptom, % [a]	Cumulative incidence of loss, %
No symptoms [a]	10	34
Cramping only	36	23
Cramping and vomiting	26	10
Cramping and bleeding	8	81
Cramping, vomiting, and bleeding	15	36

[a] No symptoms includes women without light/moderate/heavy bleeding, without vomiting, and without lower abdominal cramping

Table 3.4. Cumulative incidence of patterns of signs and symptoms by loss status and cumulative incidence of pregnancy loss by patterns of signs and symptoms

	Cumulative incidence of sign or symptom, %	Cumulative incidence of loss, %
No symptoms [a]	10	34
Cramping only	36	23
Cramping followed by vomiting, no bleeding	24	11
Cramping followed by bleeding, no vomiting	5	81
Cramping followed by vomiting followed by bleeding	6	39
Cramping followed by bleeding followed by vomiting	6	38

[a] No symptoms includes women without light/moderate/heavy bleeding, without vomiting, and without lower abdominal cramping

Table 3.5. Cox proportional hazards models showing the association between individual signs and symptoms and pregnancy loss

	Hazard Ratio (95% CI)
Any cramping versus none	0.90 (0.46, 1.77)
Any bleeding versus none	2.65 (1.70, 4.14)
Light/moderate/heavy bleeding versus none/spotting only	3.62 (2.29, 5.74)
Moderate/heavy bleeding versus none/spotting/light bleeding	4.22 (2.53, 6.69)
Any nausea/vomiting versus none	0.93 (0.34, 2.56)
Nausea only versus none	1.26 (0.45, 3.51)
Any vomiting versus none	0.63 (0.22, 1.81)
Any vomiting versus none/nausea only	0.51 (0.30, 0.86)
Any vomiting versus nausea only	0.50 (0.29, 0.85)

Table 3.6. Cox proportional hazards models showing the association between combinations of signs and symptoms and pregnancy loss

	Hazard Ratio (95% CI)
No bleeding, no cramping, no vomiting [a]	1.00 (Referent)
Cramping only	0.69 (0.30, 1.59)
Cramping + Vomiting	0.27 (0.10, 0.78)
Cramping + Bleeding	5.03 (2.07, 12.20)
Cramping + Bleeding + Vomiting	1.21 (0.51, 2.89)
Cramping only	1.00 (Referent)
Cramping + Vomiting	0.39 (0.15, 1.03)
Cramping + Bleeding	7.26 (3.52, 14.98)
Cramping + Bleeding + Vomiting	1.75 (0.86, 3.56)
Cramping + Bleeding	1.00 (Referent)
Cramping only	0.14 (0.07, 0.28)
Cramping + Vomiting	0.05 (0.02, 0.15)
Cramping + Bleeding + Vomiting	0.24 (0.11, 0.56)

[a] No bleeding, no cramping, no vomiting includes women without light/moderate/heavy bleeding, without vomiting, and without lower abdominal cramping

Table 3.7. Cox proportional hazards models showing the association between patterns of signs and symptoms and pregnancy loss

	Hazard Ratio (95% CI)
No bleeding, no cramping, no vomiting [a]	1.00 (Referent)
Cramping only	0.69 (0.30, 1.59)
Cramping followed by vomiting, no bleeding	0.28 (0.10, 0.82)
Cramping followed by bleeding, no vomiting	5.01 (1.84, 13.67)
Cramping followed by bleeding followed by vomiting	1.44 (0.46, 4.52)
Cramping followed by vomiting followed by bleeding	1.29 (0.44, 3.78)
Cramping only	1.00 (Referent)
Cramping followed by vomiting, no bleeding	0.41 (0.15, 1.11)
Cramping followed by bleeding, no vomiting	7.24 (3.04, 17.23)
Cramping followed by bleeding followed by vomiting	2.08 (0.75, 5.81)
Cramping followed by vomiting followed by bleeding	1.86 (0.73, 4.71)
Cramping followed by bleeding, no vomiting	1.00 (Referent)
Cramping only	0.14 (0.06, 0.33)
Cramping followed by vomiting, no bleeding	0.06 (0.02, 0.18)
Cramping followed by bleeding followed by vomiting	0.29 (0.09, 0.96)
Cramping followed by vomiting followed by bleeding	0.26 (0.08, 0.79)

[a] No bleeding, no cramping, no vomiting includes women without light/moderate/heavy bleeding, without vomiting, and without lower abdominal cramping

Figure 3.1. Flowchart for the study population

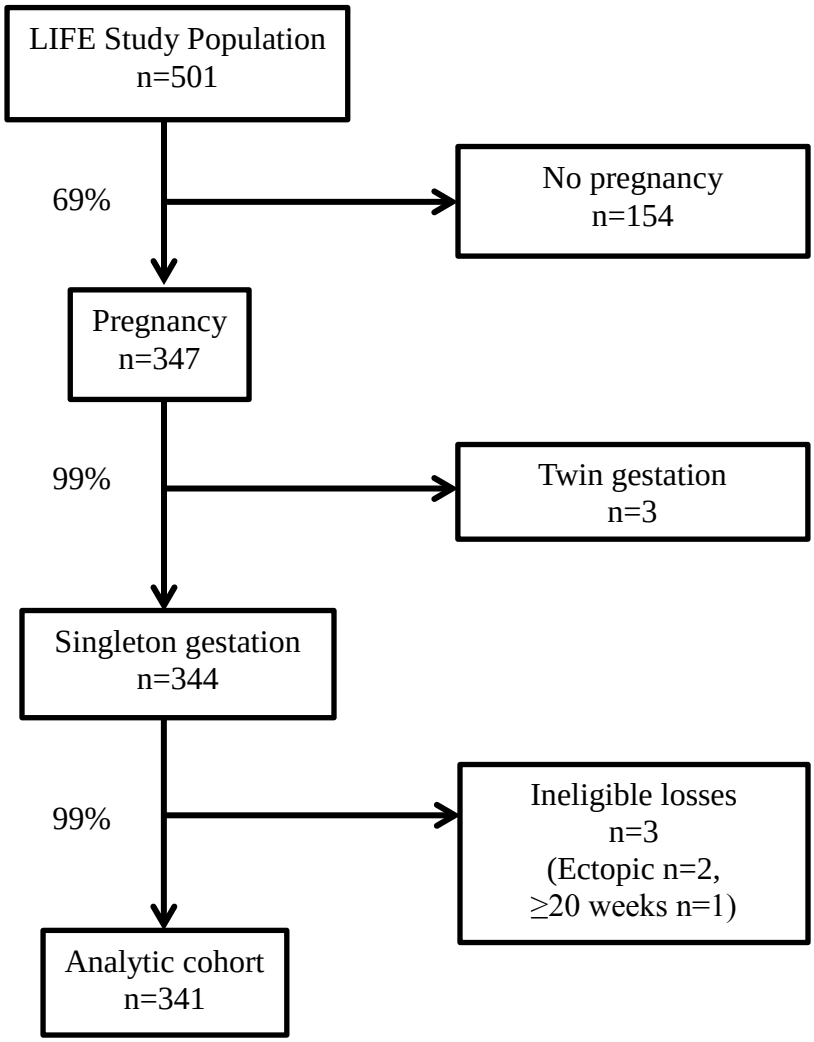


Figure 3.2. Cumulative incidence of pregnancy loss, overall, estimate and 95% confidence intervals

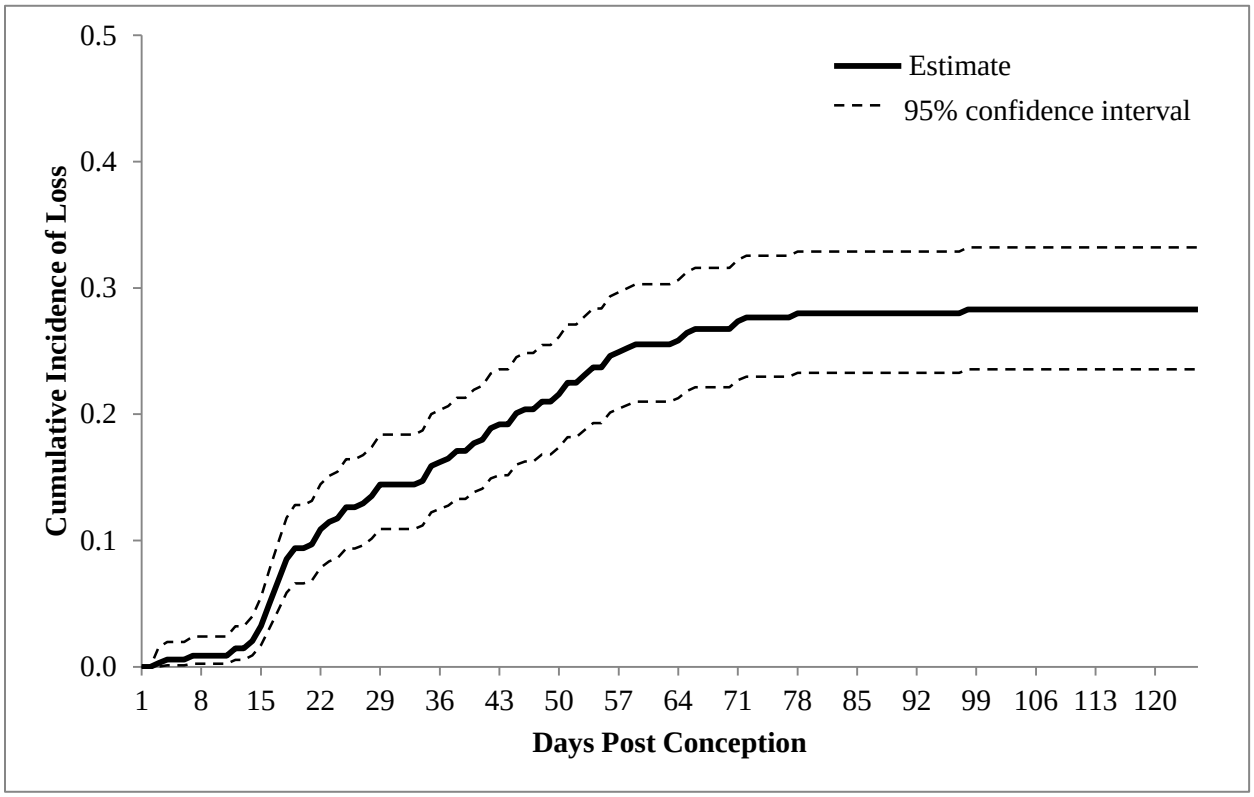


Figure 3.3. Cumulative incidence of pregnancy loss, by cramping status estimates and 95% confidence intervals

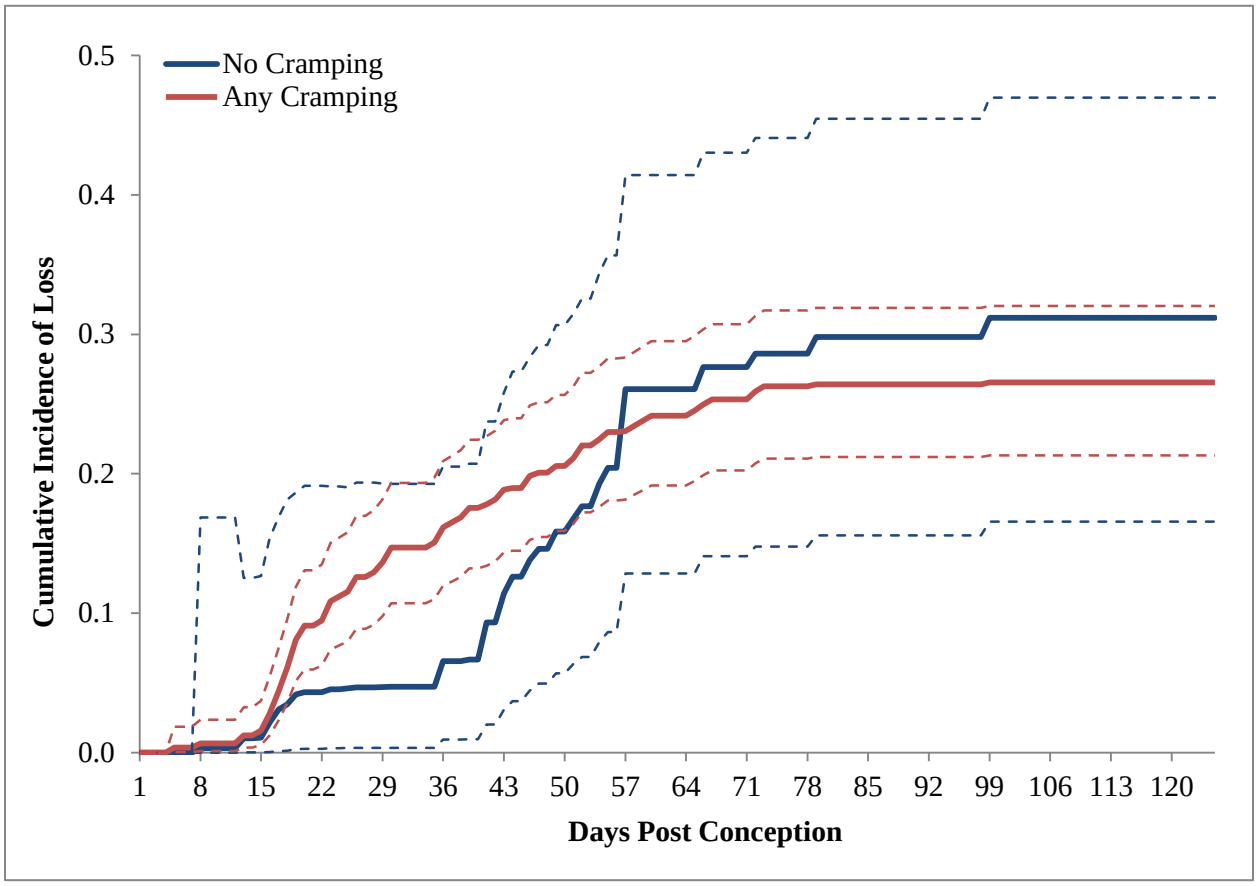
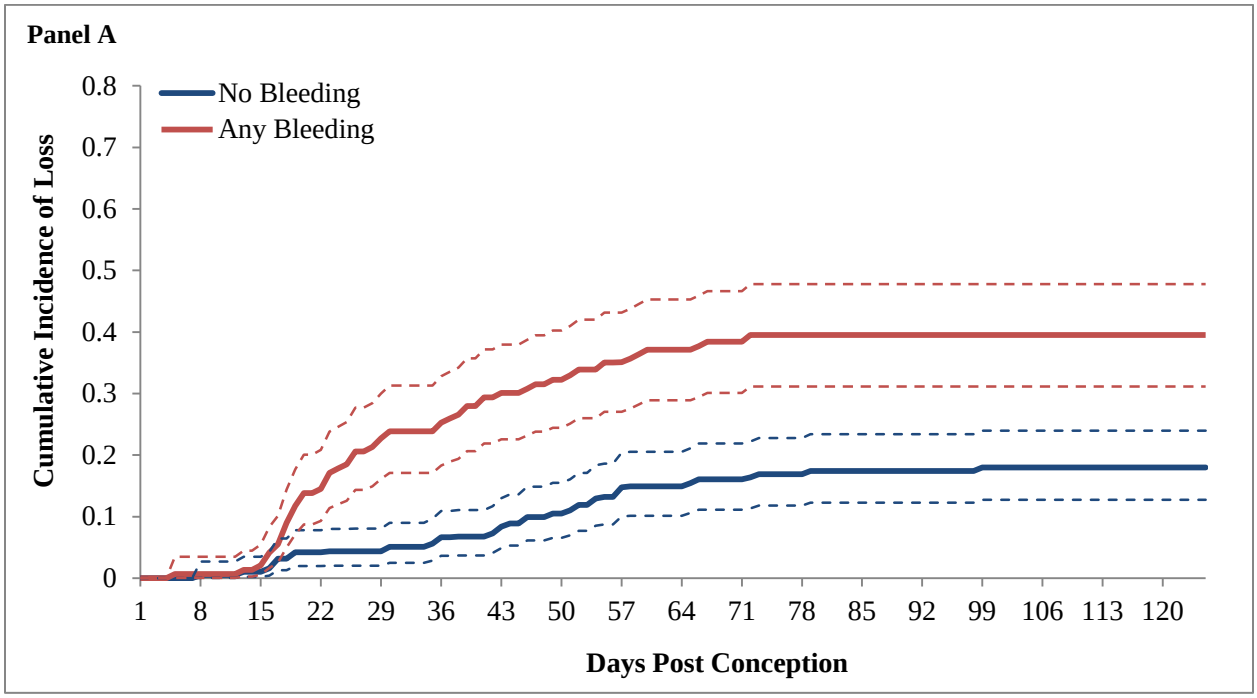


Figure 3.4. Cumulative incidence of pregnancy loss by intensity of bleeding, estimates and 95% confidence intervals



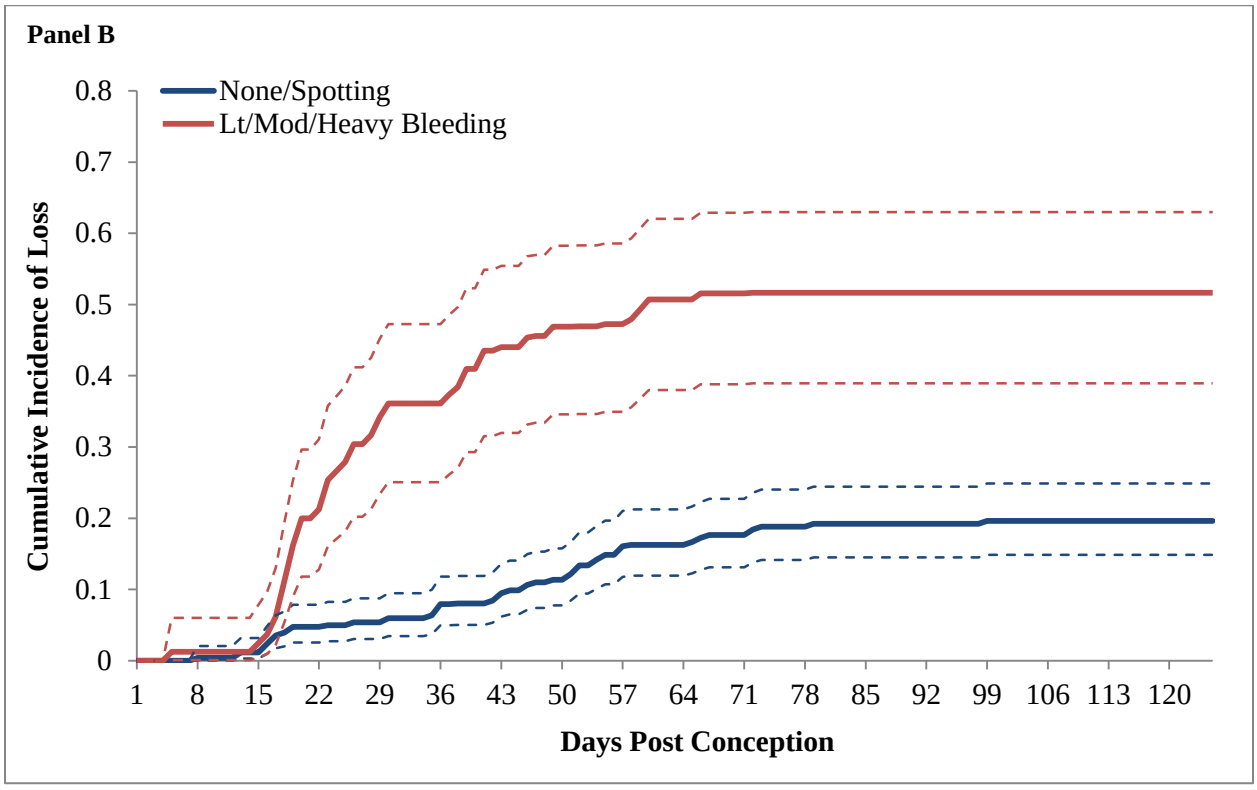


Figure 3.4. Cumulative incidence of pregnancy loss by intensity of bleeding, estimates and 95% confidence intervals (cont.)

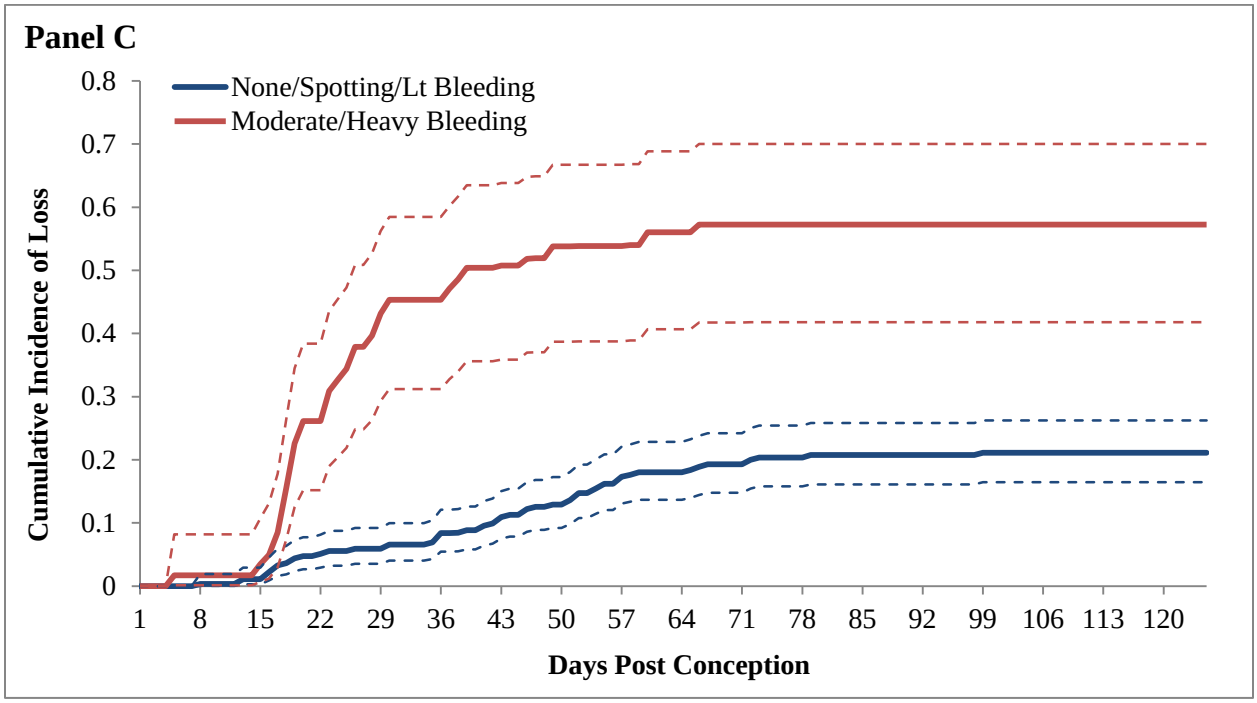
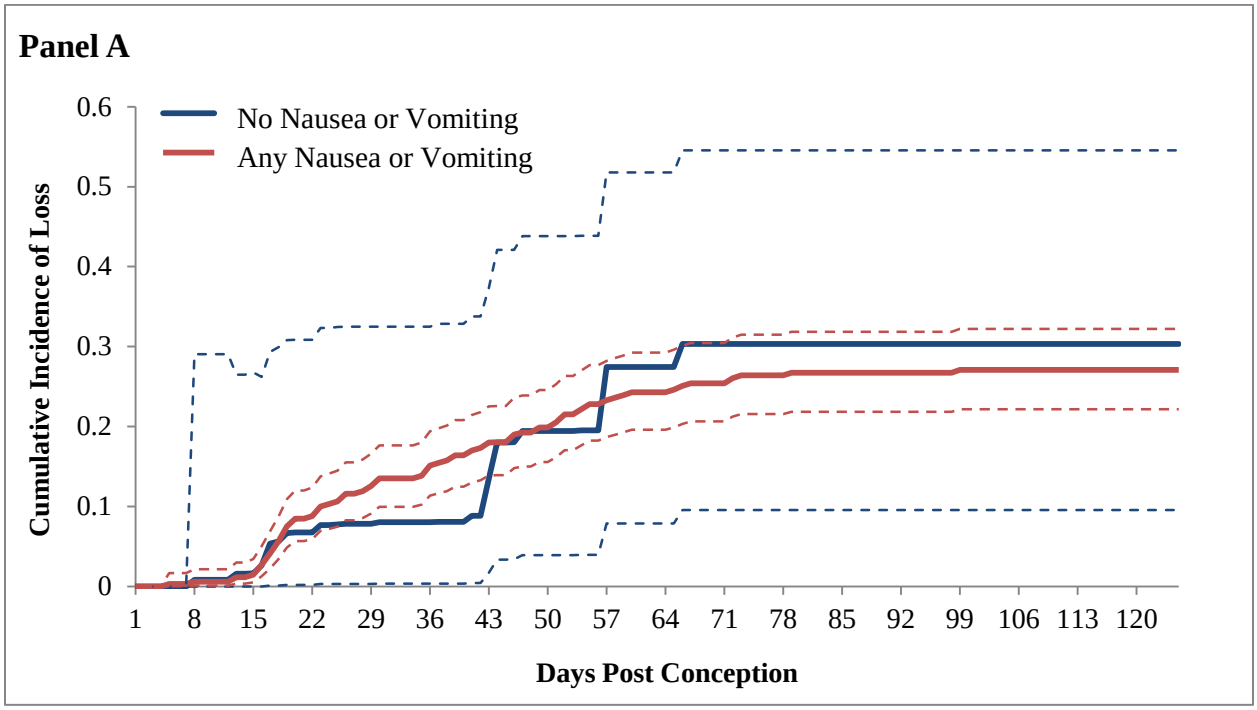


Figure 3.5. Cumulative incidence of loss by severity of nausea/vomiting, estimates and 95% confidence intervals



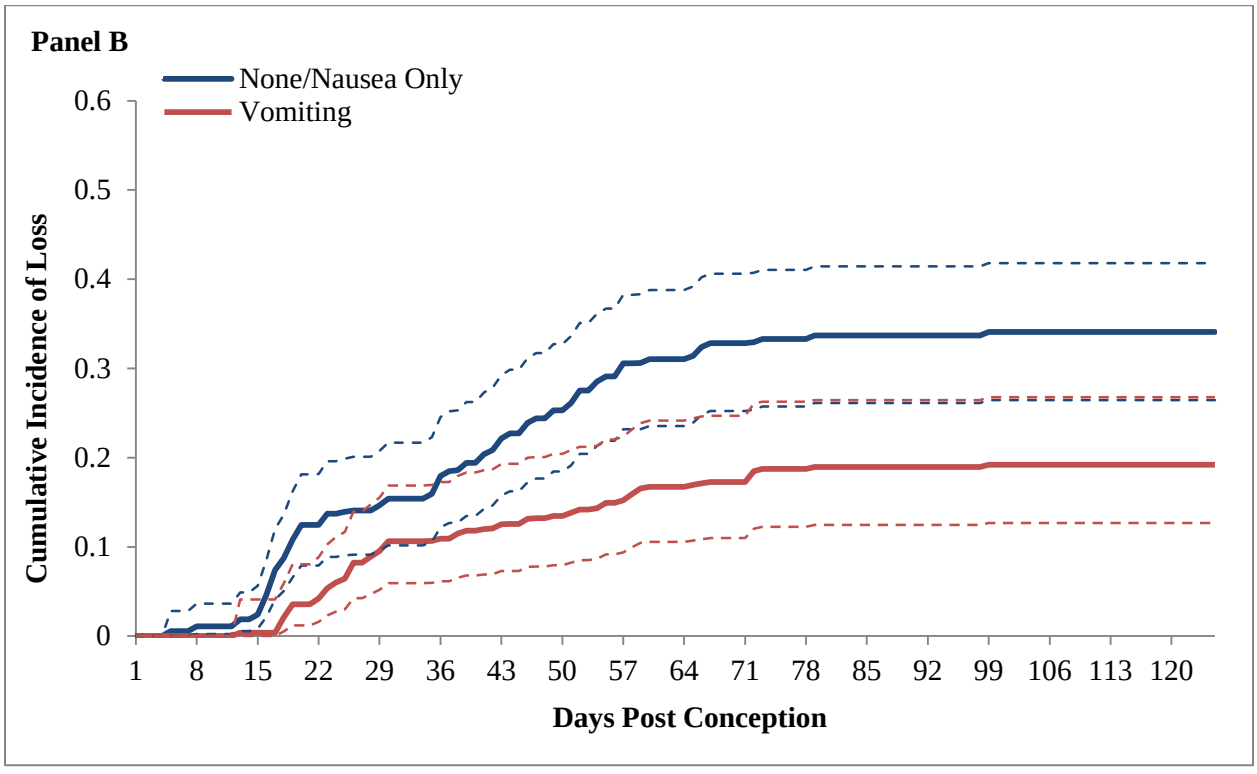


Figure 3.5. Cumulative incidence of loss by severity of nausea/vomiting, estimates and 95% confidence intervals (cont.)

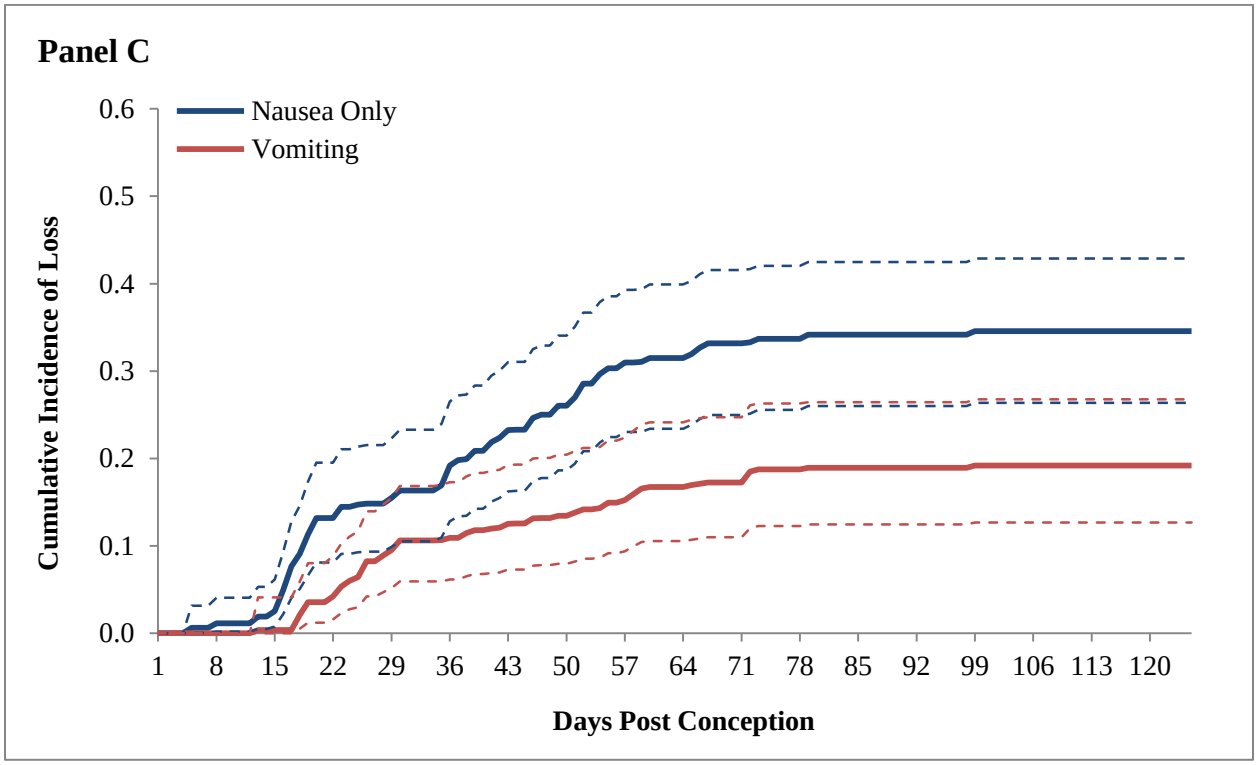


Figure 3.6. Cumulative incidence of loss by combinations of signs and symptoms, estimates

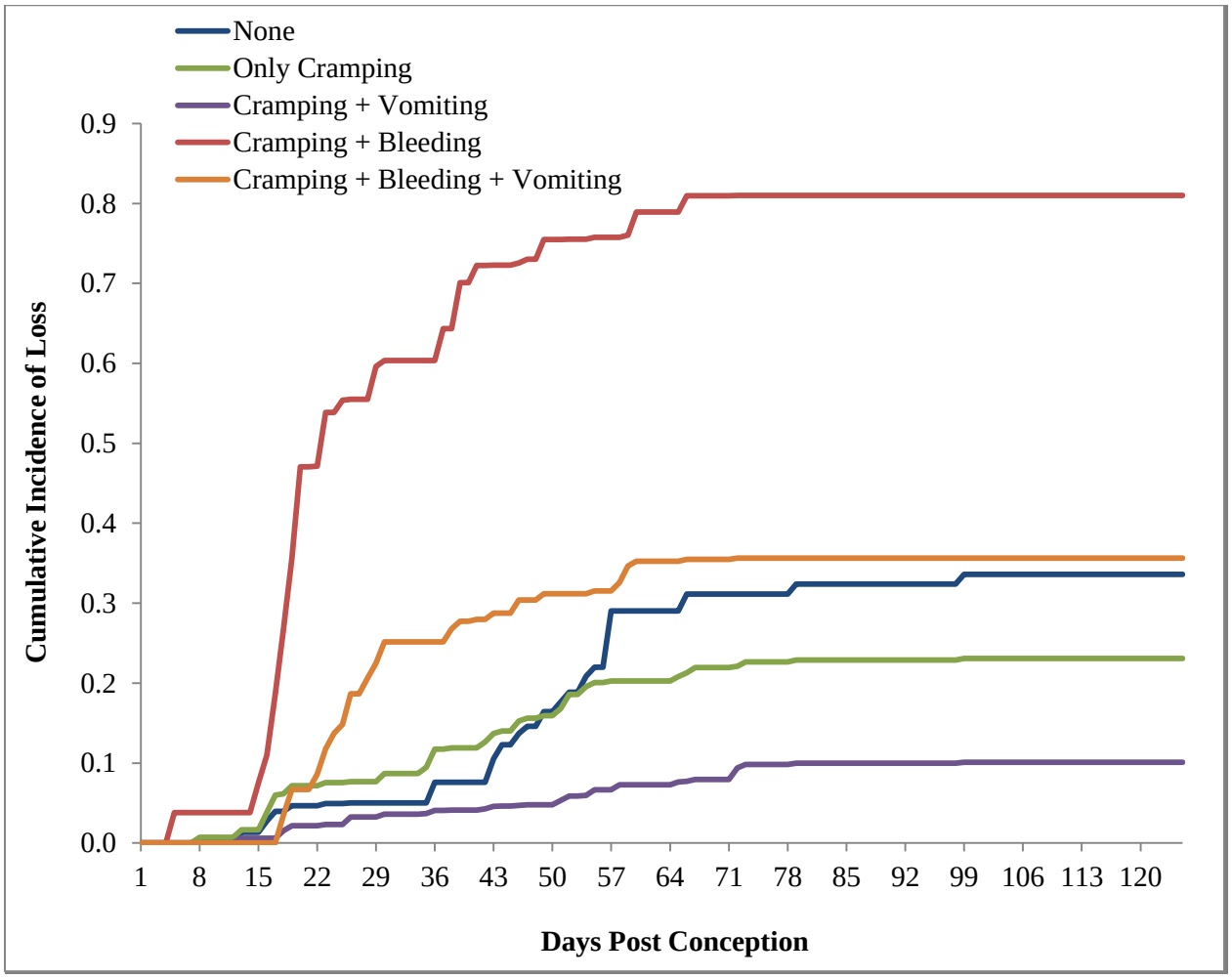
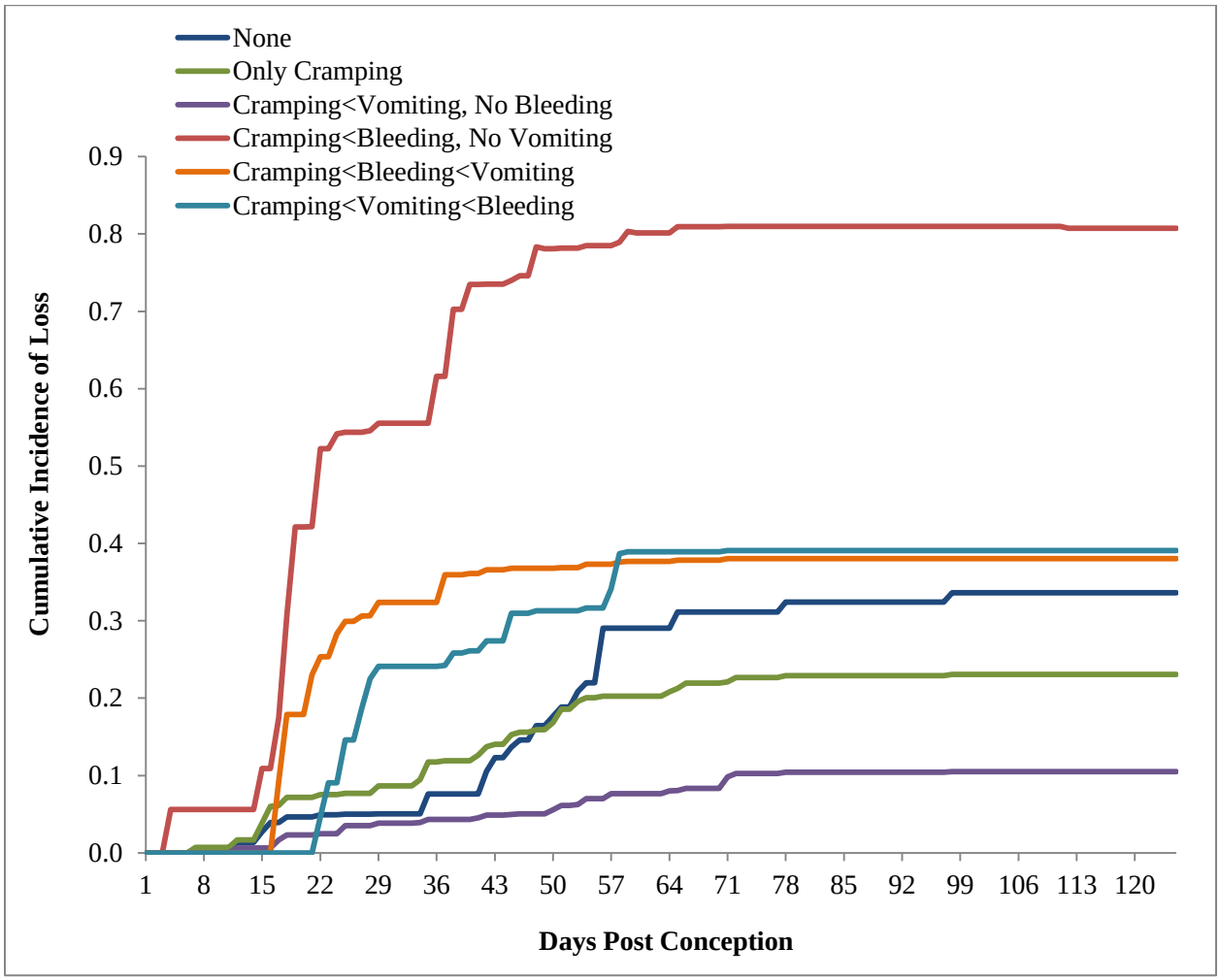


Figure 3.7. Cumulative incidence of loss by and patterns of signs and symptoms, estimates



CHAPTER 4: TIME-VARYING EFFECTS OF SIGNS AND SYMPTOMS OF EARLY PREGNANCY LOSS: FINDINGS FROM A POPULATION-BASED PRECONCEPTION COHORT

ABSTRACT

Pregnancy loss affects one-third of pregnancies, often causing psychological trauma to women and their partners; however, the signs and symptoms of pregnancy loss across gestation have yet to be fully described. Given the dynamic nature of maternal physiologic adaptation to early pregnancy progression, I posited that the relationships between signs and symptoms and subsequent pregnancy loss may change across gestational weeks. In a preconception cohort with daily follow-up, I evaluated the effects of weekly time-varying signs and symptoms on early pregnancy loss (n=95) in Cox proportional hazards models among 341 pregnancies ascertained using home pregnancy tests. The relationships between signs and symptoms and loss varied during the first five weeks following pregnancy confirmation. In the first week, vaginal bleeding (hazard ratio (HR) 8.67, 95% confidence interval (CI): 4.70, 16.01) and lower abdominal cramping (HR 1.80, 95% CI: 1.22, 2.65) were associated with increased loss, while in later weeks nausea and/or vomiting were inversely associated with loss (HR range 0.63 to 0.31, all 95% CI upper bounds below 1.00). Presence of all three symptoms was associated with loss in the first week (HR 5.19, 95% CI: 2.56, 10.51) but not in later weeks. The relationships between signs and symptoms and pregnancy loss varied across early pregnancy possibly reflecting maternal adaptation to pregnancy.

INTRODUCTION

Pregnancy loss affects one in three pregnancies,[4,14] potentially causing psychological distress to women and their partners.[23,24,26] Despite the frequency and psychological trauma associated with early embryonic or fetal loss, the signs and symptoms of pregnancy loss across gestation have yet to be fully described. Doing so requires preconception studies by which the earliest losses, which constitute the majority of losses,[4,14] can be captured and the signs and symptoms preceding loss can be prospectively ascertained. Furthermore, multiple signs and symptoms of pregnancy need to be collected and analyzed in relation to pregnancy loss as signs and symptoms often co-occur.

However, the literature on the relationships between signs and symptoms and pregnancy loss is sparse, as reviewed in Chapter 2, and only includes two preconception studies.[4,49] One preconception study of 221 women relied on laboratory measures of human chorionic gonadotropin (hCG) in serial urine collections to identify pregnancies (n=198) and losses (n=62), with most losses occurring prior to recognition by the woman.[4] While presence of vaginal bleeding[38] appeared to be associated with loss and non-specific pregnancy symptoms were inversely associated with loss,[69] these associations did not achieve statistical significance likely owing to the small study size. Another study with the preconception recruitment of 585 women reported an inverse association between nausea and pregnancy loss; however, reporting on nausea occurred monthly and could occur after the loss, which was confirmed by the clinician.[49] In contemporary cohorts of women, most pregnancies are detected by the woman herself using sensitive urine-based home pregnancy tests,[70] as was the case in this preconception study of 501

couples (described below). Such early detection of pregnancy means that many pregnancy losses are also ascertained by the women themselves prior to entering clinical care. As reported in Chapter 3, I observed significant associations between vaginal bleeding and pregnancy loss, particularly when accompanied by lower abdominal cramping, and an inverse association between vomiting and pregnancy loss.

One important limitation of previous analyses on this issue, both the prior studies from the older preconception cohort studies[38,49,69] and the results presented in Chapter 3 from this preconception cohort study, was the use of fixed effect covariates and fixed effect modeling to estimate the associations between signs and symptoms and pregnancy loss. However, the relationships between signs and symptoms and pregnancy loss may change during the early pregnancy period as maternal physiology rapidly adapts to the pregnancy. Therefore, I carried out a study to estimate the relationships between signs and symptoms and pregnancy loss using time-varying covariates to model time-varying effects.

METHODS

Study population

I used data from the Longitudinal Investigation of Fertility and the Environment (LIFE) Study, a population-based preconception cohort of 501 couples residing in 16 counties in Michigan and Texas, USA, 2005-2009. The LIFE Study has been described in detail elsewhere.[71] Briefly, couples discontinuing contraception or off contraception $\leq$ 2 months in order to attempt

pregnancy were screened for eligibility. Eligibility criteria included being in a committed relationship, both partners communicated in English or Spanish, men were aged ≥18-years-old, women were aged 18-40 years-old, had menstrual cycle lengths of 21-42 days, and had not used injectable contraception within the past year. Couples in which one or both partners had physician-diagnosed infertility/sterility were ineligible. Enrolled couples were followed until a positive home pregnancy test or for 12 months of unsuccessful pregnancy attempts. After a positive home pregnancy test, women were followed until live birth or pregnancy loss. Institutional Review Board approval was obtained from all sites and all participants provided written informed consent.

Study measures

Maternal baseline characteristics

At enrollment, women were interviewed to ascertain sociodemographic, lifestyle, and reproductive health information including age, race/ethnicity, education, household income, employment status, smoking status, and previous pregnancies and pregnancy outcomes. Body mass index (BMI) was calculated from height and weight measured by study personnel using standardized protocols.[72]

Ascertainment of pregnancy

At enrollment, women were provided with the ClearBlue digital urine-based home pregnancy test kit (Inverness Medical Innovations, Waltham, MA) and multiple pregnancy test sticks for

each cycle. Women were instructed to test on the day of expected menses. If the test was positive, they were instructed to test again one week after the first positive pregnancy test. If the pregnancy test on the day of expected menses was negative, women were instructed to test again in one week or if bleeding began. These instructions were consistent with manufacturer's guidance. The pregnancy test had an advertised hCG sensitivity of 25 IU/L, though independent testing showed that it can detect lower concentrations of pure hCG and hyperglycosylated hCG, the predominant forms of hCG in early pregnancy.[83] The digital readout categorized the result into 'pregnant' or 'not pregnant' and removed subjectivity in interpreting the result. Women recorded pregnancy test results in daily journals while trying for pregnancy. A single positive pregnancy test denoted hCG pregnancy. The distribution and cumulative incidence of positive pregnancy test by cycle day are presented in Appendix E.

Ascertainment of signs and symptoms

For five weeks following the first positive home pregnancy test, women recorded daily the occurrence of vaginal bleeding and its severity (none, spotting, light, moderate, heavy) using standardized pictographs.[77] Women also recorded daily lower belly cramping (yes/no) and nausea and/or vomiting (none, nausea only, vomiting only, or both nausea and vomiting).

Ascertainment of early pregnancy loss

Pregnancy loss was ascertained by conversion of a positive pregnancy test to a negative pregnancy test, clinical confirmation of pregnancy loss, or onset of vaginal bleeding of an

intensity and pattern consistent with expulsion of the products of conception.[78] More details are provided in Appendix A. Early pregnancy loss was defined as a pregnancy loss at <20 weeks gestational age, exclusive of ectopic pregnancy.

Statistical analysis

Time-to-event

The time-to-event was measured in days following a positive pregnancy test consistent with the timing when women were queried about the onset of signs and symptoms. Losses occurring prior to day 35 (5 weeks) post positive pregnancy test were coded as events on the observed day; losses to follow-up prior to day 35 were censored on that day. Pregnancy losses occurring ≥35 days post positive pregnancy test were coded as events at day 35. Live births and losses to follow-up ≥35 days post positive pregnancy test were censored at day 35.

Days post positive pregnancy test was chosen as the time-to-event as I was interested in assessing the time-varying effects of signs and symptoms of pregnancy loss using time-varying signs and symptoms and the survival time scale needed to be consistent with the scale for the time-varying effects. Given that signs and symptoms were collected after the positive pregnancy test and not before, the time-varying effects were estimated for the first five weeks after the positive pregnancy test; thus, the survival time was also required to be time post positive pregnancy test. The units of survival time needed to be at least as fine as the units for time-varying effects. In this case, weeks were chosen for the time-varying effects, but because days

are at least as fine as weeks, I used days post positive pregnancy test for survival time. Since signs and symptoms were not collected prior to positive pregnancy test, there would have been no effect of signs and symptoms on loss during that period.

Time-varying covariates

The values for signs and symptoms varied for each of the five weeks following a positive pregnancy test. Individual signs and symptoms for each week were coded as any versus no bleeding, light/moderate/heavy bleeding versus none/spotting only, moderate/heavy bleeding versus none/spotting/light bleeding, any versus no cramping, any versus no nausea or vomiting, any vomiting versus no nausea or vomiting, nausea only versus no nausea or vomiting. Combinations of signs and symptoms co-occurring in the same week were also constructed. All combinations were explored and the five combinations with sufficient numbers for stable estimates were 1) nausea and/or vomiting, cramping, and bleeding, 2) nausea and/or vomiting and cramping, 3) nausea and/or vomiting alone, 4) cramping alone, and 5) no nausea and/or vomiting, cramping, or bleeding. The severity of bleeding included in the combinations was in two forms: any bleeding or light/moderate/heavy bleeding.

Multiple imputation of signs and symptoms

I explored the data for completeness. Despite the intensity of daily collection, the signs and symptoms data were mostly complete. Among women with ongoing pregnancy at the beginning of the week, at least one day of bleeding information was recorded for over 85% of women during the first 4 weeks following a positive pregnancy test and half of women in week 5. At

least one day of cramping and nausea/vomiting information was completed for one-quarter of women in week 1, rising to over 80% in weeks 2-4, declining to half in week 5. Any days with missing data were imputed using the multiple imputation 'mice' package in the R software,[79] and 100 imputed data sets were generated. For each sign or symptom, all available data were used for the imputation, including maternal characteristics, other days of information on the sign or symptom, and other signs or symptoms. More information is provided in Appendix B. Any imputed data occurring on or after the day of pregnancy loss or loss to follow-up were set to missing.

Cumulative probabilities of pregnancy loss

The cumulative probabilities and 95% confidence intervals (CI) of pregnancy loss by the presence and absence of individual and combinations of signs and symptoms during each week following a positive pregnancy test were estimated from Cox proportional hazards models. Analyses were carried out in each of the 100 imputed data sets with the final results obtained by combining the results of individual data sets using Rubin's rules in PROC MIANALYZE in SAS version 9.4 to provide accurate estimates for standard errors and resulting 95% CI.

Regression modeling with time-varying effects

The censoring variable was recoded as binary: event if the outcome was a loss, censored if the outcome was a live birth or loss to follow-up (non-loss). The relative hazards of pregnancy loss were estimated for presence versus absence of time-varying individual and combinations of signs

and symptoms using Cox proportional hazards models with time-varying effects. The effects of signs and symptoms on relative hazards of pregnancy loss were allowed to vary within the regression model. A beta coefficient was estimated for each of the five weeks post pregnancy test using weekly time-varying covariates described above. Analyses were carried out in each of the 100 imputed data sets with the final results obtained by combining the results of individual data sets using Rubin's rules in PROC MIANALYZE in SAS version 9.4 to provide accurate estimates for standard errors and resulting 95% CI.

RESULTS

Characteristics of analytic cohort

Of 501 couples, 347 achieved an hCG pregnancy. Three couples had twin pregnancies and were excluded. One pregnancy loss ≥20 weeks gestation and two ectopic pregnancies occurred and were also excluded leaving 341 pregnancies. Ninety-five (28%) pregnancies ended in loss, 203 (60%) in live birth, 24 (7%) in loss to follow-up before 20 weeks, and 19 (6%) in loss to follow-up ≥20 weeks gestation. The majority of women were aged 25-34 years, non-Hispanic white, and employed, with some college, and an annual household income ≥$50,000. Most were also non-smokers and overweight or obese. Of 209 women with ≥1 pregnancies before study enrollment, 87% had ≥1 prior deliveries and 33% had ≥1 prior pregnancy losses (categories not mutually exclusive as women could have had more than one prior pregnancy).

Weekly frequencies of signs and symptoms

Five pregnancy losses and one loss to follow-up occurred the day after the positive pregnancy test and did not contribute any information to the analysis. At the beginning of week 1 post positive pregnancy test, 335 pregnancies were ongoing, 300 were ongoing at week 2, 288 at week 3, 283 at week 4, and 271 at week 5. Overall, prevalence of any bleeding was fairly stable between weeks 1-5 (range 16-22%); however, moderate/heavy bleeding became less common as gestation advanced, declining from 10% in week 1 to 1% in week 5 (Table 4.1). While any nausea and/or vomiting was also fairly stable (range 64-81%) prevalence of vomiting increased from 19% in week 1 to 32% in week 5. Prevalence of lower abdominal cramping decreased from 72% in week 1 to 53% in week 5.

The prevalence of signs and symptoms differed by loss status (Figure 4.1). In comparison with women whose pregnancies did not end in a loss, women with losses were more likely to experience any bleeding, light/moderate/heavy bleeding, or moderate/heavy bleeding with markedly higher prevalence in weeks 1 and 2. Any nausea and/or vomiting was slightly more common in women whose pregnancies subsequently ended in loss than those whose did not in the first week post positive pregnancy test; however, in weeks 2-5, any nausea and/or vomiting was more common in pregnancies that did not end in a loss. Vomiting was slightly more common among pregnancies ending in loss in weeks 1 and 2 but was higher among pregnancies not ending in loss in weeks 3-5. Any cramping was slightly more common in week 1 for pregnancies ending in loss; however, in weeks 2-5, cramping was more common among pregnancies not ending in loss.

Cumulative probabilities of pregnancy loss

Cumulative probabilities of pregnancy loss, which can be interpreted as the risks of pregnancy loss, in the presence and absence of signs and symptoms each week are presented in Table 4.2. The risk of pregnancy loss in the presence of bleeding decreased from week 1 to week 5 (64% to 13%) as did the risk of pregnancy loss in the absence of bleeding (20% to 11%). The risk of pregnancy loss decreased from week 1 to week 5 in the presence of vomiting (39% to 6%) or nausea (29% to 10%) while in the absence of either the risk of pregnancy loss was increased from week 1 to week 4 (20% to 33%) before falling in week 5 (23%). The risk of pregnancy loss decreased in the presence of cramping from week 1 to week 5 (31% to 7%) while the risk of pregnancy loss in the absence of cramping was more stable from week 1 to week 5 (21% to 16%).

Cumulative probabilities of pregnancy loss by combinations of signs and symptoms each week are presented in Table 4.3. The risk of pregnancy loss in the absence of any signs or symptoms was fairly stable from week 1 to week 5 (24% to 20%). The risk of pregnancy loss in the presence of cramping only was stable from week 1 to week 4 (15% to 16%) with an increased risk of pregnancy loss in week 5 (25%), though with wide confidence intervals (0% to 53%). The risk of pregnancy loss in the presence of nausea and/or vomiting only decreased slightly from week 1 to week 5 (18% to 11%) though with overlapping confidence intervals. The risk of pregnancy loss in the presence of nausea and/or vomiting and cramping decreased from week 1 to week 5 (22% to 5%) as did the risk of pregnancy loss in the presence of nausea and/or vomiting, cramping, and bleeding (71% to 6%).

Regression modeling results

Results for individual time-varying covariates in time-varying effect regression models are presented in Table 4.4. Any versus no bleeding was associated with pregnancy loss in week 1 (hazard ratio (HR) 6.21, 95% CI: 3.79, 10.18) and week 2 (HR 2.31, 95% CI: 1.23, 4.31), but not in weeks 3-5. Thus, the hazard ratio for week 1 was significantly different from the hazard ratio for week 5 (HR 1.49, 95% CI 0.80, 2.77). Similar results were observed for light/moderate/heavy bleeding versus none/spotting (week 1 HR 8.67, 95% CI: 4.70, 16.01; week 2 HR 3.00, 95% CI: 1.41, 6.37; week 3 HR 1.42, 95% CI 0.52, 3.84) and moderate/heavy bleeding versus none/spotting/light bleeding (week 1 HR 8.27, 95% CI: 4.18, 16.36; week 2 HR 4.65, 95% CI: 2.02, 10.71; week 4 HR 0.85, 95% CI 0.22, 3.24), with hazard ratios in week 1 being significantly different from hazard ratios in week 3 or week 4. Any nausea and/or vomiting were not associated with pregnancy loss in week 1; however, it was inversely associated with loss in weeks 2-5 (HR range 0.63 to 0.31, all 95% CI below 1.00). Similar results were observed for nausea only versus no nausea and/or vomiting (HR range 0.52 to 0.36, all 95% CI below 1.00). Any vomiting versus no nausea and/or vomiting was inversely associated with loss in weeks 3-5, though the estimate for week 4 was not significant (week 3 HR 0.28, 95% CI: 0.09, 0.83; week 4 HR 0.11, 95% CI: 0.00, 10.78; week 5 HR 0.21, 95% CI: 0.07, 0.68). Any versus no cramping was associated with pregnancy loss in the first week post positive pregnancy test (HR: 1.80, 95% CI: 1.22, 2.65); however, cramping was inversely associated with loss in weeks 3-5, though the week 4 effect was not significant (week 3 HR 0.48, 95% CI: 0.26, 0.89; week 4 HR 0.60, 95% CI: 0.34, 1.03; week 5 HR 0.35, 95% CI: 0.17, 0.72).

Results for combinations of time-varying covariates in time-varying effect models are presented in Table 4.5. In the first week post positive pregnancy test, women with all three symptoms had higher rates of pregnancy loss (HR: 5.19, 95% CI: 2.56, 10.51) compared with women without any symptoms, while nausea and/or vomiting and cramping either alone or in combination with one another were not associated with loss. In weeks 2-5, nausea and/or vomiting either alone or in combination with cramping were inversely associated with loss.

DISCUSSION

In this preconception cohort, there was weekly variability in the prevalence of signs and symptoms in early pregnancy. In particular, the first week following a positive pregnancy test when approximately one-third of losses occurred was different from subsequent weeks and the variability appeared to be most marked for the more severe signs and symptoms. Specifically, moderate/heavy bleeding prevalence declined from 10% in the first week post positive pregnancy test to 1% in the fifth week, while prevalence of vomiting increased from 19% in the first week to 32% in the fifth week. The relationships between signs and symptoms and pregnancy loss also varied across weeks. The positive relationship between bleeding and pregnancy loss was most pronounced, and a positive relationship between lower abdominal cramping and pregnancy loss was observed, in the first week following a positive pregnancy test. Even in the setting of nausea and/or vomiting, bleeding with cramping was associated with increased risk of pregnancy loss in the first week. Conversely, nausea and/or vomiting were not associated with pregnancy loss in the first week following a positive pregnancy test but were inversely associated with loss in subsequent weeks. The presence of all three symptoms was

associated with loss in the first week but not in later weeks. This may reflect a particular symptomatic presentation of losses in the first week that is different from that occurring slightly later in pregnancy.

Previous studies,[38] including results presented in Chapter 3, using fixed effect modeling in preconception studies with daily follow-up showed that vaginal bleeding was more common in pregnancies ending in a loss than in those not ending in a loss. A prior pregnancy cohort study calculated week-specific probabilities of loss by bleeding status and showed the greatest probability of miscarriage occurred in the earliest weeks in pregnancies with heavy, but not light, bleeding.[37] A case series on threatened abortion in pregnancies with demonstrated fetal cardiac activity showed the prevalence of miscarriage was three times greater in weeks 5-6 than in weeks 7-20.[88] In this study, I found that vaginal bleeding was associated with pregnancy loss but only in the first two weeks post positive pregnancy test (at approximately 4-5 weeks gestation), which was consistent with the descriptive data from the pregnancy cohort[37] and case series.[88]

As reported in Chapter 3, no significant association between lower abdominal cramping and pregnancy loss was observed. However, fixed effect analyses of combinations of signs and symptoms co-occurring with cramping, showed that cramping with bleeding was associated with pregnancy loss, while cramping with vomiting was inversely associated with pregnancy loss. In contrast, using time-varying effects in this study, I found that lower abdominal cramping was positively associated with loss in the first week post positive pregnancy test and inversely associated with loss in later weeks. When considering combinations of signs and symptoms, I

corroborated findings from Chapter 3 that cramping with nausea and/or vomiting was inversely associated with loss, but only after the first week post positive pregnancy test. I also found that cramping with bleeding and nausea and/or vomiting was associated with loss in the first week but not in subsequent weeks.

As reported in Chapter 3, any vomiting during the early pregnancy period was inversely associated with pregnancy loss, while only experiencing nausea throughout the early pregnancy period was not associated with loss in fixed effect models. However, a previous preconception study with monthly reporting of nausea found that nausea was inversely associated with pregnancy loss, though nausea in that study was not distinguished from nausea with vomiting.[49] Using time-varying effects in this Chapter, I found that vomiting was inversely associated with loss after the first and second weeks post positive pregnancy test, which corroborated the findings from fixed effects models in prior studies. However, I found experiencing nausea only during a given week was also inversely associated with pregnancy loss after the first week, which was in contrast to findings from fixed effects models in Chapter 3. Of note, in the time-varying effects analysis of this Chapter, women could report nausea only in one week but then report vomiting in another week whereas in the fixed covariate, fixed effect models if a woman reported vomiting at any time during the five week early pregnancy period, she would have been categorized in the vomiting group (applicable to about half of women with nausea). Thus, the totality of the exposure (e.g., nausea only) over the first few weeks of early pregnancy was more informative for pregnancy loss than individual weeks of exposure as weekly information does not capture precedent or subsequent exposures (e.g., vomiting).

This study had several strengths. Firstly, the preconception design facilitated the prospective capture of signs and symptoms relative to pregnancy loss ascertainment. This minimized the potential recall bias that could arise if signs and symptoms were elicited after the loss (i.e., exposure misclassification differential by outcome). Secondly, by capturing signs and symptoms daily, I was able to evaluate these factors as time-varying covariates with time-varying effects on pregnancy loss. Thirdly, by ascertaining pregnancies using sensitive home pregnancy tests, pregnancies and pregnancy losses were captured early in gestation. This extended the relationship between signs and symptoms and pregnancy loss to very early pregnancy and thus increased the applicability of the results to contemporary cohorts of pregnant women who have information from early pregnancy testing.[4,14] It also facilitated the evaluation the time-varying relationships between signs and symptoms of pregnancy loss during the period prior to clinical care entry.

Despite these strengths, these findings must be interpreted in light of the study's limitations. Firstly, by study design daily information on signs and symptoms were collected only through the first five weeks following a positive pregnancy test; therefore, I was unable to evaluate the weekly time-varying effects of signs and symptoms on loss beyond that period. As this corresponds with the end of organogenesis and the beginning of the fetal period, signs and symptoms in relation to loss may differ from the early pregnancy period. Secondly, despite being the largest prospective preconception study with daily follow-up to date,[82] there were few pregnancy losses beyond the first trimester. Thirdly, I was unable to examine all possible combinations of signs and symptoms owing to reduced statistical power. Finally, 7% of pregnancies were lost to follow-up <20 weeks gestation, though I used a survival analytic

censoring approach to estimate precise incidence rates.

CONCLUSIONS

The relationships between signs and symptoms and pregnancy loss were dynamic across early pregnancy in this preconception cohort study. In the first week following a positive pregnancy test, bleeding and cramping were associated with loss even in the setting of nausea and/or vomiting. However, new relationships between signs and symptoms emerged after the second week and appeared relatively stable, with nausea and/or vomiting inversely associated and bleeding no longer associated with pregnancy loss. Symptomatic presentation of pregnancy loss varied by week, and this has clinical implications if corroborated in future preconception studies. Specifically, vaginal bleeding after approximately 6 weeks gestation may not necessitate an immediate evaluation. While these results suggest that vaginal bleeding before 6 weeks is associated with a higher rate of pregnancy loss and that nausea and/or vomiting are indicative of a positive response to pregnancy, the maternal physiologic responses to early pregnancy, causes of signs and symptoms and their relation to early pregnancy loss require more basic science, clinical, and epidemiologic research. Developing prognostic models for pregnancy loss inclusive of time-varying signs and symptoms will be useful for women and clinicians in terms of understanding physiologic and pathologic processes associated with early pregnancy and pregnancy loss.

Table 4.1. Prevalence of signs and symptoms by week following positive pregnancy test, overall and by pregnancy loss status

	Overall , % (n=335)	No Loss, % (n=245)	Loss, % (n=90)
No symptoms			
Week 1	13	13	12
Week 2	18	15	27
Week 3	14	13	20
Week 4	11	8	24
Week 5	13	12	25
Any bleeding			
Week 1	20	13	39
Week 2	16	14	24
Week 3	18	17	24
Week 4	19	18	29
Week 5	22	22	25
Light/moderate/heavy bleeding			
Week 1	13	7	29
Week 2	10	8	18
Week 3	8	8	11
Week 4	11	10	15
Week 5	10	10	9
Moderate/heavy bleeding			
Week 1	10	6	23
Week 2	7	5	17
Week 3	4	4	5
Week 4	6	7	6
Week 5	1	0	3

Table 4.1. Prevalence of signs and symptoms by week following positive pregnancy test, overall and by pregnancy loss status (cont.)

	Overall , % (n=335)	No Loss, % (n=245)	Loss, % (n=90)
Any nausea and/or vomiting			
Week 1	75	72	81
Week 2	64	66	55
Week 3	78	81	62
Week 4	81	86	54
Week 5	81	83	58
Any vomiting			
Week 1	19	17	24
Week 2	14	14	18
Week 3	19	21	9
Week 4	22	25	6
Week 5	32	34	17
Nausea only [a]			
Week 1	74	72	80
Week 2	58	62	42
Week 3	72	75	55
Week 4	72	76	49
Week 5	67	70	50
Any cramping			
Week 1	72	69	77
Week 2	56	57	52
Week 3	48	51	29
Week 4	50	52	40
Week 5	53	56	33

[a] Nausea, but not vomiting, during the week

Table 4.2. Cumulative probabilities of pregnancy loss by occurrence of signs and symptoms each week following positive pregnancy test

	Presence Probability of loss (95% confidence interval)	Absence Probability of loss (95% confidence interval)
Any bleeding		
Week 1	0.64 (0.48, 0.79)	0.20 (0.16, 0.25)
Week 2	0.31 (0.16, 0.46)	0.16 (0.12, 0.21)
Week 3	0.22 (0.10, 0.33)	0.14 (0.10, 0.18)
Week 4	0.23 (0.11, 0.34)	0.12 (0.08, 0.17)
Week 5	0.13 (0.04, 0.22)	0.11 (0.07, 0.15)
Light/moderate/heavy bleeding		
Week 1	0.78 (0.59, 0.96)	0.22 (0.17, 0.27)
Week 2	0.40 (0.18, 0.62)	0.17 (0.12, 0.21)
Week 3	0.22 (0.03, 0.41)	0.15 (0.11, 0.19)
Week 4	0.21 (0.05, 0.37)	0.14 (0.10, 0.18)
Week 5	0.11 (0.00, 0.23)	0.11 (0.08, 0.15)
Moderate/heavy bleeding		
Week 1	0.76 (0.55, 0.98)	0.23 (0.18, 0.28)
Week 2	0.56 (0.27, 0.85)	0.16 (0.12, 0.21)
Week 3	0.22 (0.00, 0.53)	0.15 (0.11, 0.19)
Week 4	0.14 (0.00, 0.32)	0.14 (0.10, 0.18)
Week 5	0.39 (0.00, 0.70)	0.11 (0.07, 0.15)
Any nausea and/or vomiting		
Week 1	0.31 (0.24, 0.38)	0.20 (0.10, 0.30)
Week 2	0.16 (0.11, 0.21)	0.23 (0.15, 0.30)
Week 3	0.12 (0.08, 0.16)	0.27 (0.16, 0.37)
Week 4	0.10 (0.06, 0.14)	0.33 (0.22, 0.45)
Week 5	0.08 (0.05, 0.12)	0.23 (0.12, 0.35)

Table 4.2. Cumulative probabilities of pregnancy loss by occurrence of signs and symptoms each week following positive pregnancy test (cont.)

	Presence Probability of loss (95% confidence interval)	Absence Probability of loss (95% confidence interval)
Any vomiting		
Week 1	0.39 (0.19, 0.58)	0.20 (0.10, 0.30)
Week 2	0.25 (0.10, 0.40)	0.23 (0.15, 0.30)
Week 3	0.08 (0.00, 0.15)	0.27 (0.16, 0.37)
Week 4	0.04 (0.00, 0.11)	0.33 (0.22, 0.45)
Week 5	0.06 (0.00, 0.12)	0.23 (0.12, 0.35)
Any nausea [a]		
Week 1	0.29 (0.21, 0.37)	0.20 (0.10, 0.30)
Week 2	0.14 (0.08, 0.19)	0.23 (0.15, 0.30)
Week 3	0.13 (0.08, 0.18)	0.27 (0.16, 0.37)
Week 4	0.12 (0.07, 0.16)	0.33 (0.22, 0.45)
Week 5	0.10 (0.04, 0.15)	0.23 (0.12, 0.35)
Any cramping		
Week 1	0.31 (0.24, 0.38)	0.21 (0.11, 0.30)
Week 2	0.17 (0.11, 0.23)	0.20 (0.13, 0.26)
Week 3	0.09 (0.05, 0.14)	0.20 (0.14, 0.26)
Week 4	0.12 (0.06, 0.17)	0.17 (0.11, 0.23)
Week 5	0.07 (0.03, 0.12)	0.16 (0.10, 0.22)

[a] Nausea, but not vomiting, during the week

Table 4.3. Cumulative probabilities of pregnancy loss by occurrence of combinations of signs and symptoms each week following positive pregnancy test

	Probability of loss (95% confidence interval)
No symptoms [a]	
Week 1	0.24 (0.09, 0.38)
Week 2	0.26 (0.16, 0.37)
Week 3	0.22 (0.10, 0.33)
Week 4	0.30 (0.16, 0.45)
Week 5	0.20 (0.07, 0.33)
Cramping only	
Week 1	0.15 (0.02, 0.32)
Week 2	0.15 (0.05, 0.26)
Week 3	0.15 (0.00, 0.32)
Week 4	0.16 (0.00, 0.36)
Week 5	0.25 (0.00, 0.53)
Nausea/vomiting only	
Week 1	0.18 (0.03, 0.33)
Week 2	0.13 (0.05, 0.21)
Week 3	0.17 (0.10, 0.24)
Week 4	0.10 (0.04, 0.16)
Week 5	0.11 (0.03, 0.18)
Nausea/vomiting + cramping	
Week 1	0.22 (0.14, 0.29)
Week 2	0.13 (0.06, 0.20)
Week 3	0.07 (0.02, 0.13)
Week 4	0.08 (0.02, 0.14)
Week 5	0.05 (0.00, 0.11)

Table 4.3. Cumulative probabilities of pregnancy loss by occurrence of combinations of signs and symptoms each week following positive pregnancy test (cont.)

	Probability of loss (95% confidence interval)
Nausea/vomiting + cramping + bleeding	
Week 1	0.71 (0.54, 0.88)
Week 2	0.31 (0.14, 0.49)
Week 3	0.11 (0.00, 0.22)
Week 4	0.14 (0.02, 0.25)
Week 5	0.06 (0.00, 0.14)

[a] No symptoms includes women without any cramping, bleeding, nausea and/or vomiting

Table 4.4. Time-varying effects of individual signs and symptoms on pregnancy loss by week following positive pregnancy test

Sign or Symptom	Hazard Ratio	95% Confidence Interval
Any versus no bleeding		
Week 1	6.21	3.79, 10.18
Week 2	2.31	1.23, 4.31
Week 3	1.49	0.80, 2.77
Week 4	1.57	0.88, 2.79
Week 5	0.84	0.41, 1.73
Light/moderate/heavy bleeding versus none/spotting		
Week 1	8.67	4.70, 16.01
Week 2	3.00	1.41, 6.37
Week 3	1.42	0.52, 3.84
Week 4	1.38	0.59, 3.24
Week 5	0.63	0.18, 2.20
Moderate/heavy bleeding versus none/spotting/light bleeding		
Week 1	8.27	4.18, 16.36
Week 2	4.65	2.02, 10.71
Week 3	1.36	0.28, 6.52
Week 4	0.85	0.22, 3.24
Week 5	2.84	0.71, 11.36
Any versus no cramping		
Week 1	1.80	1.22, 2.65
Week 2	0.92	0.58, 1.46
Week 3	0.48	0.26, 0.89
Week 4	0.60	0.34, 1.03
Week 5	0.35	0.17, 0.72

Table 4.4. Time-varying effects of individual signs and symptoms on pregnancy loss by week following positive pregnancy test (cont.)

Sign or Symptom	Hazard Ratio	95% Confidence Interval
Any versus no nausea and/or vomiting		
Week 1	1.33	0.88, 1.99
Week 2	0.63	0.40, 0.99
Week 3	0.46	0.30, 0.70
Week 4	0.37	0.22, 0.60
Week 5	0.31	0.18, 0.52
Nausea only [a] versus no nausea/vomiting		
Week 1	1.21	0.77, 1.91
Week 2	0.52	0.32, 0.87
Week 3	0.52	0.33, 0.80
Week 4	0.44	0.26, 0.73
Week 5	0.36	0.19, 0.68
Vomiting versus no nausea/vomiting		
Week 1	1.72	0.82, 3.60
Week 2	1.03	0.47, 2.24
Week 3	0.28	0.09, 0.83
Week 4	0.11	0.00, 10.78
Week 5	0.21	0.07, 0.68

[a] Nausea, but not vomiting, during the week

Table 4.5. Time-varying effects of combinations of signs and symptoms on pregnancy loss by week following positive pregnancy test

Combination of Signs and Symptoms	Hazard Ratio [a]	95% Confidence Interval	Hazard Ratio [b]	95% Confidence Interval
Week 1				
Cramping only	0.49	0.12, 1.97	0.53	0.13, 2.19
Nausea/vomiting only	0.62	0.23, 1.72	0.68	0.24, 1.93
Nausea/vomiting + cramping	0.94	0.56, 1.59	0.87	0.49, 1.54
Nausea/vomiting + cramping + bleeding	5.19	2.56, 10.51	4.48	2.36, 8.50
Week 2				
Cramping only	0.55	0.24, 1.25	0.60	0.26, 1.38
Nausea/vomiting only	0.44	0.22, 0.91	0.50	0.23, 1.07
Nausea/vomiting + cramping	0.46	0.24, 0.92	0.50	0.25, 1.03
Nausea/vomiting + cramping + bleeding	1.60	0.67, 3.83	1.35	0.59, 3.07
Week 3				
Cramping only	0.44	0.12, 1.69	0.58	0.15, 2.19
Nausea/vomiting only	0.60	0.36, 1.00	0.65	0.38, 1.13
Nausea/vomiting + cramping	0.24	0.10, 0.56	0.27	0.11, 0.65
Nausea/vomiting + cramping + bleeding	0.48	0.11, 2.15	0.39	0.11, 1.43
Week 4				
Cramping only	1.01	0.37, 2.72	0.62	0.14, 2.75
Nausea/vomiting only	0.35	0.17, 0.70	0.38	0.19, 0.78
Nausea/vomiting + cramping	0.27	0.12, 0.62	0.28	0.11, 0.70
Nausea/vomiting + cramping + bleeding	0.54	0.16, 1.81	0.52	0.19, 1.45

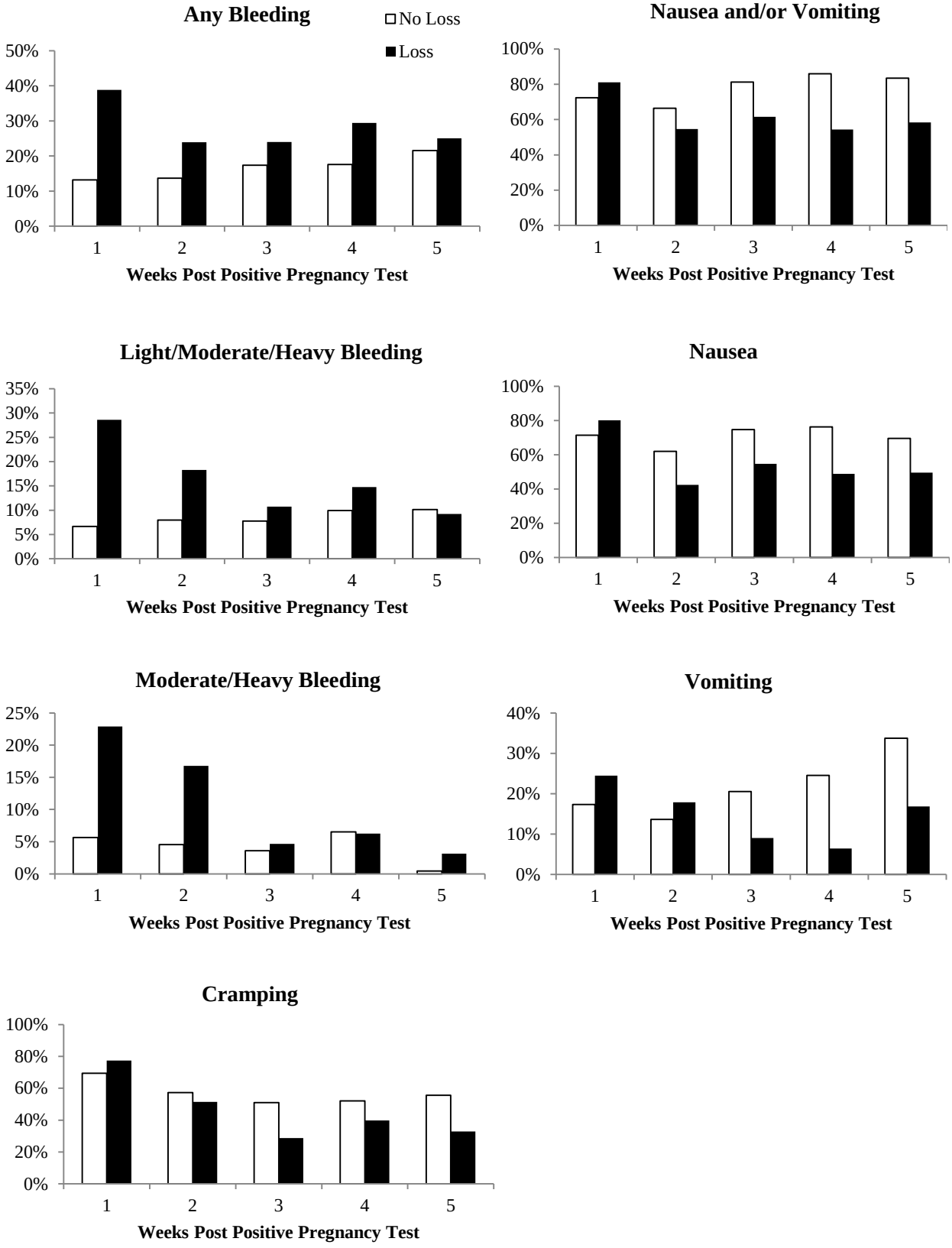
Table 4.5. Time-varying effects of combinations of signs and symptoms on pregnancy loss by week following positive pregnancy test (cont.)

Combination of Signs and Symptoms	Hazard Ratio [a]	95% Confidence Interval	Hazard Ratio [b]	95% Confidence Interval
Week 5				
Cramping only	0.88	0.22, 3.52	0.98	0.24, 4.04
Nausea/vomiting only	0.41	0.19, 0.88	0.40	0.17, 0.91
Nausea/vomiting + cramping	0.20	0.07, 0.52	0.19	0.06, 0.61
Nausea/vomiting + cramping + bleeding	0.01	0.00, 229	0.21	0.05, 0.95

[a] Reference includes women with no symptoms or with spotting only

[b] Reference includes women with no symptoms

Figure 4.1. Weekly prevalence of signs and symptoms by pregnancy loss status



CHAPTER 5: CONCLUSIONS

SUMMARY OF SPECIFIC AIMS

I addressed three specific aims in this dissertation in order to more completely describe the natural history of pregnancy loss. First, I systematically reviewed the existing literature on the associations between signs and symptoms of pregnancy and subsequent pregnancy loss. Second, I used a population-based preconception cohort in the USA with daily prospective ascertainment of signs and symptoms from two to seven weeks post-conception to assess the relationships between multiple signs and symptoms during early pregnancy and subsequent pregnancy loss in the first 20 weeks gestation using a fixed covariate and fixed effect survival analytic approach. Third, I used the same preconception cohort and information on signs and symptoms but employed a time-varying covariate and time-varying effect survival analytic approach to determine if the relationships between multiple signs and symptoms during early pregnancy and subsequent pregnancy loss were constant across gestational age.

SUMMARY OF FINDINGS

During my systematic review of the literature, I identified two preconception and 16 pregnancy cohort studies that attempted to ascertain signs and symptoms prior to pregnancy loss. These studies were conducted in several different countries from the 1950s until the 2010s; however, most studies, including the two preconception studies, only examined relationships between individual signs and symptoms and pregnancy loss. Two pregnancy cohort studies examined the relationships between multiple signs and symptoms and pregnancy loss, but they date from the

1950s when pregnancy recognition occurred much later in gestation than currently. A more recent study examined nausea in the setting of vaginal bleeding but only among women presenting with threatened abortion. From this systematic review, some relationships between signs and symptoms and pregnancy loss were fairly consistent: vaginal bleeding, particularly heavier bleeding, was associated with increased risk of loss, while vomiting, and in some studies nausea also, was inversely associated with pregnancy loss even in the setting of vaginal bleeding. However, notable data gaps were identified. First, since many of the existing studies were pregnancy cohorts, the earliest pregnancy losses, which constituted the majority of losses, were not included. Second, the relationships between multiple signs and symptoms and pregnancy loss were limited and evaluations of temporal ordering of signs and symptoms and pregnancy loss were absent. Third, only one pregnancy cohort study evaluated time-varying probabilities of pregnancy loss by timing of vaginal bleeding. These data gaps served as the impetus for the second and third aims of this dissertation.

In the second aim, I described the cumulative incidence of several individual signs and symptoms, as well as combinations and temporal patterning of signs and symptoms, (namely vaginal bleeding, lower abdominal cramping, nausea, and vomiting) during the earliest period of pregnancy from two to seven weeks post-conception in a preconception cohort. I then described the cumulative incidence of pregnancy loss by the presence of individual, combinations, and patterns of signs and symptoms during early pregnancy. Finally, using a fixed covariate and fixed effect survival modeling approach, I estimated the associations between individual, combinations, and patterns of signs and symptoms and pregnancy loss <20 weeks gestation. I found that lower abdominal cramping was common in early pregnancy, though it was not

associated with pregnancy loss absent other signs and symptoms. Vomiting and nausea were also common in early pregnancy. The experience of vomiting, but not nausea alone, was associated with a lower incidence of loss. While vaginal bleeding was less common in early pregnancy, it was associated with increased incidence of loss, particularly if accompanied by lower abdominal cramping, though if vomiting subsequently followed bleeding the incidence of loss was lower than if vomiting remained absent.

In the third aim, I evaluated the weekly associations between individual and combinations of signs and symptoms and pregnancy loss in the first five weeks after a positive home pregnancy test, approximately two to seven weeks post-conception, using weekly time-varying covariates and time-varying effects in a survival analytic model. I found that the weekly prevalence of signs and symptoms was fairly stable for most signs and symptoms though more variation was noted for the more severe symptoms (e.g., vomiting and moderate/heavy vaginal bleeding); however, the relationships between signs and symptoms and pregnancy loss varied across early pregnancy. The first week after pregnancy discovery appeared to be particularly vulnerable to pregnancy loss in the setting of vaginal bleeding and lower abdominal cramping, even if nausea and/or vomiting were present; however, in the second through fifth weeks, bleeding accompanied by cramping, nausea and/or vomiting was no longer associated with loss. In contrast, nausea and/or vomiting, alone or in combination with lower abdominal cramping, were inversely associated with loss in the second through fifth weeks, but not in the first week.

There were many strengths underlying this work. The first was the use of information from a preconception cohort with prospective daily follow-up of women from the beginning of their

pregnancy attempt. Second, the use of sensitive urine-based home pregnancy tests facilitated the capture of the earliest pregnancies and pregnancy losses occurring prior to presentation for clinical care. Since very early losses comprise the majority of pregnancy loss, this increased the direct applicability of my findings to early pregnancy losses, particularly those in the first trimester. Third, the daily capture of signs and symptoms from two to seven weeks post-conception minimized the possibility for recall bias in the reporting of signs and symptoms, and potential bias in the observed associations between signs and symptoms and pregnancy loss. The daily capture of information also facilitated the evaluation of patterns and temporal ordering of signs and symptoms, in relation to pregnancy loss, which was another novel contribution to the literature. Finally, the daily capture of information on signs and symptoms allowed for the use of time-varying covariates and the estimation of time-varying effect, which revealed that the relationships between signs and symptoms and pregnancy loss changed during early pregnancy, another novel finding.

There were also some limitations to this work. First, despite being the largest preconception study with daily follow-up, there were few pregnancy losses after 14 weeks gestation, limiting the amount of information and applicability of findings to losses in the second trimester. Second, by design, data on signs and symptoms were collected daily only from two to seven weeks post-conception. Therefore, the weekly time-varying effects of signs and symptoms on loss could only be estimated for this time period, which corresponds to the embryonic period. Finally, there was a small (7%) loss to follow-up in the study before 20 weeks gestation. However, survival analytic censoring approaches were used to estimate effects with appropriate precision despite the loss to follow-up.

In light of these strengths and limitations, I will comment on the internal and external validity of this work. I believe that the analytic aims are internally valid; they are not hindered by information bias, selection bias, or confounding. While women were prompted daily as to the presence or absence of multiple signs and symptoms, I have no reason to believe that women would respond to these prompts differently by their eventual loss status. Indeed, the prospective ascertainment of signs and symptoms relative to loss ascertainment is a strength of these data that mitigates information bias (*e.g.*, measurement error). While there was some loss to follow-up, all available data was included in the analysis using survival analytic techniques; this mitigates selection bias due to loss to follow-up. While this descriptive work was designed to be inclusive of a broad range of couples achieving pregnancy, and thus, no *a priori* plan was established to examine signs and symptoms of pregnancy loss within subgroups of women (*e.g.*, by parity or maternal age), I did examine several maternal characteristics as possible confounding variables of the association between signs and symptoms and loss. None of these characteristics met statistical criteria for inclusion as confounding variables in the statistical models, and the existing literature does not firmly support the inclusion of any of these variables as *a priori* confounding variables in the models. Therefore, I do not believe that confounding has affected this study.

With regards to the external validity, or generalizability, of these findings, I believe these findings apply to pregnancies achieved spontaneously (*e.g.*, without the use of assisted reproductive technologies). The LIFE Study was designed to be inclusive of a broad range of

couples attempting pregnancy; therefore, exclusion criteria were minimal. The LIFE Study was also population-based to be generalizable to the population of couples attempting pregnancy. While the LIFE Study, as with all studies in the US relying upon volunteers, had a study population that was better educated, had higher income, and was less racially/ethnically diverse than the US population, I do not believe that differences in income, education, or race/ethnicity would impact the relationship between signs and symptoms and pregnancy loss. It is possible that unprompted reporting of signs and symptoms may differ by these characteristics; however, women were prompted daily on their signs and symptoms. From the perspective of putative biological mechanisms, there is no evidence to suggest that the underlying relationships between signs and symptoms and pregnancy loss would differ by these characteristics. I would not extend these findings to women with pregnancies achieved through assisted reproductive technologies because these women receive exogenous hormones prior to and during pregnancy. As various maternal hormones are believed to be associated with the presence of multiple signs and symptoms, it may be that the relationship between signs and symptoms and pregnancy loss are different among women receiving exogenous hormones than among women not receiving these hormones (*e.g.*, spontaneously achieved pregnancies).

SIGNIFICANCE OF FINDINGS

In this dissertation, I have described some novel relationships between signs and symptoms and pregnancy loss. The absence of signs and symptoms was not associated with an appreciable difference in pregnancy loss incidence. However, the presence of individual signs and symptoms and combinations of signs and symptoms was associated with an increased or decreased

incidence of pregnancy loss. The absence of signs and symptoms was not as informative for pregnancy loss risk as the presence of one or more specific symptoms or signs. Furthermore, this work demonstrated that the time period over which signs and symptoms did or did not occur was an important consideration for estimating pregnancy loss incidence.

The novel findings from the two distinct, but complimentary, analytic approaches have implications for the epidemiologic, clinical, and basic science communities as they suggested that the inference of findings from a fixed effect model using a fixed covariate covering a wide exposure window (e.g., effect of bleeding in early pregnancy on pregnancy loss) may be different from the inference of findings from a time-varying effect model using time-varying covariates over shorter intervals of exposure (e.g., effect of bleeding immediately following a positive pregnancy test versus bleeding several weeks after a positive pregnancy test on pregnancy loss). While time-varying effect modeling may be preferred for assessing sensitive windows of exposure relevant for organogenesis (e.g., differences in effects by gestational week of exposure), it requires a larger number of subjects than fixed effect modeling. Furthermore, one may have data limited by uncertain gestational dating or queries on coarse exposure windows necessitating the use of a fixed effect model that will provide an average effect of the exposure on the outcome. In some instances, fixed covariates may be preferred to time-varying covariates as they provide a summary measure of exposure over a longer period that cannot be captured with time-varying covariates. One approach is not necessarily better than the other as they answer different questions; consequently, they also have different implications for public health messaging. Thus, one must be careful with the interpretation of the results. The findings from the

two analytic aims highlighted these considerations; the significance for each of the three signs and symptoms are described below.

Lower abdominal cramping has received limited attention in previous work and has only been evaluated in the context of vaginal bleeding. I found that lower abdominal cramping was common in early pregnancy and occurred by itself and in combination with nausea and/or vomiting and vaginal bleeding. In the absence of other signs or symptoms, lower abdominal cramping was not associated with pregnancy loss. However, when cramping was associated with vaginal bleeding, the cumulative incidence of loss was higher than for any other combination of signs and symptoms in the early pregnancy period. When examining the weekly time-varying effects of signs and symptoms, cramping with vaginal bleeding, even in the presence of nausea and/or vomiting, was associated with increased risk of pregnancy loss, though only in the first week after pregnancy discovery. In contrast, cramping co-occurring with vomiting was associated with the lowest cumulative incidence of loss of all combinations of signs and symptoms. In weekly time-varying effects analyses, cramping with nausea and/or vomiting was associated with decreased risk of loss, though only after the first week post pregnancy discovery. Together, these findings suggested that lower abdominal cramping may be the norm rather than the exception in early pregnancy. Furthermore, cramping alone was not a harbinger of loss and more information on other signs and symptoms is needed to better understand the risk of loss in pregnancies where cramping is present.

Vaginal bleeding, particularly more severe bleeding, was associated with an increased risk of

pregnancy loss; however, vaginal bleeding often co-occurred with one or more other signs or symptoms, which provided greater information on the risk of loss. In women with vaginal bleeding and lower abdominal cramping, the subsequent occurrence of vomiting resulted in a lower cumulative incidence of pregnancy loss when compared with pregnancy loss among women in whom vomiting did not occur. In weekly time-varying effects models, bleeding most often occurred with cramping and nausea and/or vomiting. In these models, the presence of all three symptoms was associated with an increased risk of loss only during the first week post pregnancy discovery. These findings suggested that the presence of vaginal bleeding was most likely to be associated with pregnancy loss soon after pregnancy discovery and that the co-occurrence of nausea and/or vomiting reduced the risk of loss in the setting of vaginal bleeding only somewhat later in gestation after the period of the greatest loss incidence had passed.

By evaluating nausea and/or vomiting as both fixed effects and time-varying effects, I found two different but complimentary associations with pregnancy loss that provided more insight into the relationship of nausea and vomiting and loss during early pregnancy. Under the fixed covariate and fixed effect modeling, I found that vomiting, but not nausea alone, was associated with a lower cumulative incidence of pregnancy loss. However, in the weekly time-varying covariate and time-varying effect models, I found that after the first week post pregnancy discovery both vomiting, and nausea alone were associated with decreased risks of pregnancy loss. This may be due to the different exposure definitions used in the two models. In the fixed effect models, a woman may have had nausea alone for several weeks. However, if she had just one day of vomiting at any point during the early pregnancy period, she would have been classified as having vomiting for the entire period. In the time-varying covariate models, however, a woman

may have had several weeks of nausea only and then had a single week with vomiting. Her weeks of nausea alone would have been evaluated as nausea alone and her later week of vomiting would have counted as vomiting for the single week. Together, these findings suggested that the totality of the exposure (e.g., nausea only without any vomiting) during the early pregnancy period may have been more informative for pregnancy loss than individual weeks of exposure.

This work points to areas for further exploration in epidemiologic, clinical, and basic science research. First, the prompt for lower abdominal cramping in this study, 'lower belly cramping,' may capture a heterogeneous symptom with possibly heterogeneous causes. For example, cramping consistent with menstrual-like cramps may reflect uterine contraction early in gestation associated with expulsion of the products of conception. Alternatively, later in gestation, less severe lower abdominal cramping of a different character may result from rising estrogen levels that are associated both with a healthy ongoing pregnancy and uterine contractility. Future work in epidemiologic studies should seek to better document and grade the severity and typology of lower abdominal cramping in order to better understand its relation to pregnancy loss. Clinical and basic science studies may focus on more directly observing uterine contractility during early pregnancy via ultrasound or other measures to better capture the physiologic response of the uterus to implantation and subsequent embryonic growth or demise. Second, the findings from time-varying effect models of vaginal bleeding and pregnancy loss provide evidence that the symptomatology of pregnancy loss may differ by gestational age and that these differences occur quite early in gestation, as soon as the first week after pregnancy discovery or roughly two weeks post-conception. This may be particularly important information for women and clinicians trying

to identify an impending pregnancy loss. It may also be important for epidemiologic researchers designing measures in preconception and pregnancy studies as using one global variable to assess a sign or symptom over an entire trimester may result in missing important associations within smaller time intervals. Third, the findings on nausea and vomiting in relation to loss highlighted that the evaluation of an exposure over a longer time period may provide different inferences than the evaluation of an exposure over shorter time intervals in relation to an outcome. As our understanding of the causes of embryonic development and demise is still in its infancy, we should continue to explore a wide range of exposure periods in our analyses but collect data in smaller time intervals since we can collapse smaller intervals into larger ones but not vice versa.

FUTURE DIRECTIONS

The findings of this dissertation suggest that developing prognostic models for pregnancy loss by incorporating multiple time-varying signs and symptoms may be useful for women and clinicians to address the questions regarding the risk of an early pregnancy loss. However, future studies utilizing prospectively collected daily data are needed to corroborate these findings and extend our understanding of signs and symptoms and pregnancy loss at the population level. More work also is needed to understand signs and symptoms in relation both to maternal characteristics, such as hormonal profiles and demographic and lifestyle characteristics, and fetal characteristics, such as sex and embryo quality.

Specifically, future studies should evaluate whether the natural history of pregnancy loss observed in this population-based cohort of spontaneously achieved pregnancies is replicated in other populations of spontaneous achieved pregnancies and pregnancies achieved through assisted reproductive technologies. Given the expense of conducting preconception cohort studies, existing data, such as that collected at the daily level by mobile applications designed to track signs and symptoms associated with the menstrual cycle and pregnancy, may be leveraged to determine if the findings observed here are replicated among other populations. Furthermore, these data sources often collect more signs and symptoms than those queried in this study, for example, breast tenderness, smell and taste aversions, fatigue. Thus, future studies could extend the work completed in this dissertation on the natural history of pregnancy loss. More signs and symptoms could also potentially improve the predictive value of prognostic models if they are found to be associated with pregnancy loss.

Pregnancies achieved through assisted reproductive technologies offer unique opportunities to examine biomarkers and possible biological mechanisms that underlie the signs and symptoms of pregnancy loss. For example, the quality of embryos can be assessed in relation to the appearance of signs and symptoms and their relationships with pregnancy loss. Furthermore, since women with pregnancies achieved through assisted reproductive technologies are closely observed at multiple clinic visits during early pregnancy and are known to be highly compliant with their medical care, quantitative values of serum and urinary hCG, as well as serum and urinary levels of progesterone, estrogen, and their metabolites, over the course of early pregnancy can be assessed in relation to the appearance of signs and symptoms and their relationships with pregnancy loss. These studies could support or refute the theories that high

progesterone and hCG levels are associated with vomiting while high estrogen levels are associated with lower abdominal cramping and provide new insights into the hormonal basis of the symptomatology of early pregnancy and pregnancy loss.

Future work is also needed by clinical and basic scientists to increase our understanding of the physiologic processes underlying (mal)adaption to pregnancy, in particular, the physiology of lower abdominal cramping. Given the time-varying effects of lower abdominal cramping in relation to pregnancy loss observed in this study, lower abdominal cramping likely has heterogeneous typology and heterogeneous causes. Using ultrasound, uterine contractility can be observed and correlated with hormonal profiles and other uterine features (*e.g.*, presence of subchorionic hematoma, uterine fibroids) to better understand the relationship between lower abdominal cramping and pregnancy loss.

This dissertation work serves as a much-needed foundation for exploring the complex relationships among maternal and fetal characteristics (*e.g.*, maternal hormones and embryo quality), multiple and varied signs and symptoms of early pregnancy, and pregnancy loss. It appears that concerning and reassuring patterns of signs and symptoms of early pregnancy loss do exist, yet their biologic mechanisms remain to be fully elucidated. Future work should focus on the interplay of hormonal and physiologic adaptions to early pregnancy, symptomatology, and pregnancy loss to further our understanding of the natural history of pregnancy loss.

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APPENDICES

Appendix A: Definition and dating of pregnancy loss

Pregnancy loss is identified using the following definition:

Following a single positive pregnancy test and in the absence of a subsequent live birth,

1) Diagnostic test

2) Saw a doctor for loss

3) Bleeding pattern consistent with expulsion of products of conception

Diagnostic test includes negative pregnancy test, ultrasound indicated fetus died, or heartbeat not detected. Saw a doctor for loss is presumably to confirm loss by diagnostic test. Bleeding pattern consistent with expulsion of products of conception is ≥2 consecutive days of bleeding where ≥2 consecutive days are light, moderate, or heavy bleeding) in the absence of a diagnostic test/doctor visit consistent with the amount of blood loss expected during the expulsion of products of conception.[78]

For this dissertation, early pregnancy loss was defined as a pregnancy loss (described above) occurring <20 weeks gestation (dating described below) and exclusive of ectopic pregnancies.

The date of pregnancy loss ascertainment using the following method:

1) Diagnostic test: Date of diagnostic test confirming loss.

2) Saw a doctor for loss: Date of seeing a doctor for loss.

3) Bleeding pattern: Date of the midpoint of the bleeding episode consistent with expulsion of products of conception.

The first day of the bleeding episode is the first day of light, moderate, or heavy bleeding and the last day of the episode is the last day of any bleeding. The midpoint of the bleeding episode is used to avoid bias associated with assigning either the first or the last day of the episode as the date of ascertainment of loss. This approach is commonly used in interval censoring to avoid bias. With other methods of ascertainment, a woman may have a pregnancy loss ascertained at any point during the bleeding episode.

Appendix B: Multiple imputation model

The multiple imputation models included the several maternal characteristics to inform the daily values for signs and symptoms. Note that in the multiple imputation models, maternal characteristics were imputed first if missing based on other maternal characteristics; thus, the imputation for signs and symptoms were based on full information on maternal characteristics. Recruitment site, employment status, smoking status, age category, history of gynecologic problem, body mass index category, race/ethnicity, education, income, history of pregnancy loss, parity, other days of information on the same sign or symptoms, and all days of other signs and symptoms.

Appendix C: Maternal characteristics by pregnancy outcome

Female Characteristics by Pregnancy Outcome (n=341)

	Loss (n=95)	Live Birth (n=203)	LTF <20 wks (n=24)	LTF ≥20 wks (n=19)	
	n (%)	n (%)	n (%)	n (%)	p-value
Age					
<25 years old	5 (20)	18 (72)	1 (4)	1 (4)	0.02
25-29 years old	42 (27)	87 (55)	17 (11)	12 (8)	
30-34 years old	29 (25)	79 (69)	3 (3)	3 (3)	
35+ years old	19 (43)	19 (43)	3 (7)	3 (7)	
Race/Ethnicity					
Non-Hispanic White	80 (28)	166 (59)	23 (8)	14 (5)	0.47
Non-Hispanic Black	3 (50)	2 (50)	0 (0)	0 (0)	
Hispanic	7 (24)	19 (66)	1 (3)	2 (7)	
Other	4 (20)	13 (65)	0 (0)	3 (15)	
Education					
High school or less	6 (40)	9 (60)	0 (0)	0 (0)	0.43
Some college or more	88 (27)	192 (60)	23 (7)	19 (6)	
Income					
<$50,000	11 (25)	29 (66)	3 (7)	1 (2)	0.41
$50-99,999	51 (32)	87 (54)	14 (9)	9 (6)	
$100,000+	29 (23)	82 (65)	7 (6)	9 (7)	
Employed					
No	20 (29)	37 (54)	4 (6)	7 (10)	0.26
Yes	75 (27)	166 (61)	20 (7)	12 (4)	
Body mass index					
<18.5	2 (40)	3 (60)	0 (0)	0 (0)	0.54
18.5-24.9	41 (25)	101 (62)	15 (9)	7 (4)	
25.0-29.9	23 (26)	53 (60)	4 (5)	8 (9)	
30.0+	29 (35)	45 (54)	5 (6)	4 (5)	

Female Characteristics by Pregnancy Outcome (cont.)

	Loss (n=95)	Live Birth (n=203)	LTF <20 wks (n=24)	LTF ≥20 wks (n=19)	
	n (%)	n (%)	n (%)	n (%)	p-value
Site					
Michigan	18 (28)	42 (65)	5 (8)	0 (0)	0.18
Texas	77 (28)	161 (58)	19 (7)	19 (7)	
Prior delivery					
No prior pregnancy	36 (27)	81 (61)	10 (8)	5 (4)	0.43
No prior delivery	7 (26)	13 (48)	4 (15)	3 (11)	
Prior delivery	51 (28)	108 (60)	10 (6)	10 (6)	
Prior loss					
No prior pregnancy	36 (27)	81 (61)	10 (8)	5 (4)	0.23
No prior loss	34 (24)	81 (58)	12 (9)	12 (9)	
Prior loss	24 (35)	40 (59)	2 (3)	2 (3)	
Smoker at enrollment					
No	88 (28)	189 (59)	23 (7)	18 (6)	0.94
Yes	7 (30)	14 (61)	1 (4)	1 (4)	
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	p-value
Cycle day of positive pregnancy test	29 (26, 32)	29 (27, 32)	31 (28, 36)	28 (26, 36)	0.29

LTF: loss to follow-up

Appendix D: Maternal characteristics by method of loss ascertainment

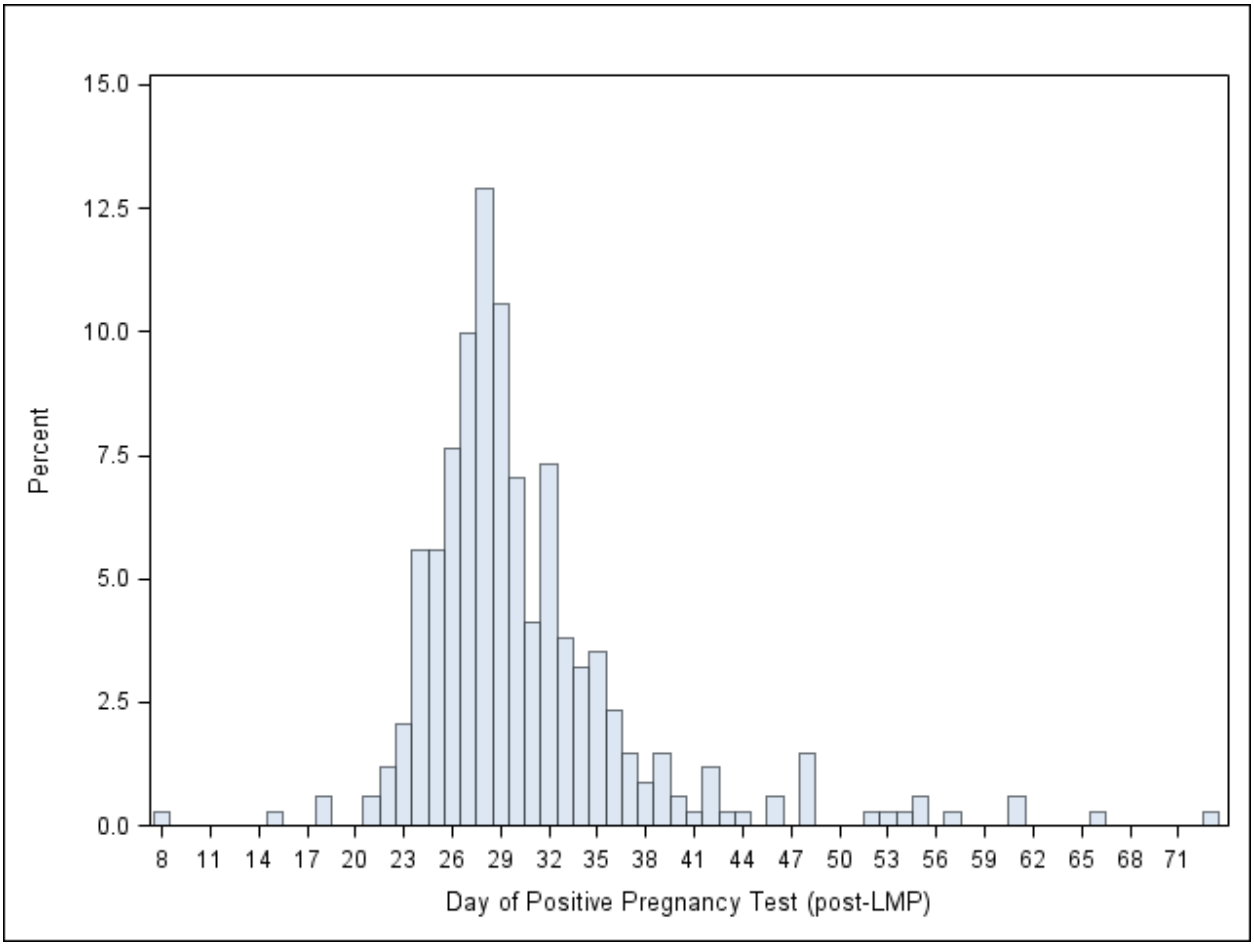
Female Characteristics by Method of Loss Ascertainment (n=95)

	Bleeding pattern (n=11)	Heartbeat not detected (n=30)	Negative pregnancy test (n=32)	Ultrasound indicated demise (n=6)	Saw a doctor (n=16)	
	n (%)	n (%)	n (%)	n (%)	n (%)	p-value
Age						
<25 years old	0 (0)	0 (0)	2 (40)	0 (0)	3 (60)	0.17
25-29 years old	6 (14)	10 (24)	15 (36)	5 (12)	6 (14)	
30-34 years old	2 (7)	12 (41)	10 (34)	0 (0)	5 (17)	
35+ years old	3 (16)	8 (42)	5 (26)	1 (6)	2 (11)	
Race/Ethnicity						
White	11 (14)	23 (29)	28 (35)	5 (6)	13 (16)	0.75
Black	0 (0)	1 (33)	1 (33)	1 (33)	0 (0)	
Hispanic	0 (0)	3 (43)	2 (29)	0 (0)	2 (29)	
Other	0 (0)	2 (50)	1 (25)	0 (0)	1 (25)	
Education						
≤ High school	0 (0)	0 (0)	3 (50)	1 (17)	2 (33)	0.26
≥ Some college	11 (13)	29 (33)	29 (33)	5 (6)	14 (16)	
Income						
<$50,000	0 (0)	1 (9)	6 (55)	1 (9)	3 (27)	0.15
$50-99,999	8 (16)	15 (29)	14 (27)	5 (10)	9 (18)	
$100,000+	2 (7)	12 (41)	12 (41)	0 (0)	3 (10)	
Employed						
No	0 (0)	5 (25)	9 (45)	1 (17)	5 (25)	0.26
Yes	11 (15)	25 (33)	23 (31)	5 (83)	7 (15)	
Body mass index						
<18.5	1 (50)	0 (0)	0 (0)	0 (0)	1 (50)	0.40
18.5-24.9	6 (15)	9 (22)	16 (39)	3 (7)	7 (17)	
25.0-29.9	3 (13)	9 (39)	5 (22)	1 (4)	5 (22)	
30.0+	1 (3)	12 (41)	11 (38)	2 (7)	3 (10)	

Female Characteristics by Method of Loss Ascertainment (cont.)

	Bleeding pattern (n=11)	Heartbeat not detected (n=30)	Negative pregnancy test (n=32)	Ultrasound indicated demise (n=6)	Saw a doctor (n=16)	
	n (%)	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**p-value**
Site						
Michigan	5 (28)	7 (39)	4 (22)	1 (6)	1 (6)	0.09
Texas	6 (8)	23 (30)	28 (36)	5 (6)	15 (19)	
Prior delivery						
No prior	5 (14)	11 (31)	11 (31)	3 (8)	6 (17)	0.98
No prior delivery	1 (14)	3 (43)	2 (29)	0 (0)	1 (14)	
Prior delivery	4 (8)	16 (31)	19 (37)	3 (6)	9 (18)	
Prior loss						
No prior	5 (14)	11 (31)	11 (31)	3 (8)	6 (17)	0.95
No prior loss	4 (12)	10 (29)	13 (38)	2 (6)	5 (15)	
Prior loss	1 (4)	9 (38)	8 (33)	1 (4)	5 (21)	
Smoker at enrollment						
No	10 (91)	29 (97)	28 (88)	6 (100)	15 (94)	0.64
Yes	1 (9)	1 (3)	4 (13)	0 (0)	1 (6)	
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**p-value**
Cycle day of positive pregnancy test	28 (27, 33)	29 (26, 32)	29 (6, 32)	31 (27, 33)	29 (28, 33)	0.95
Cycle day of loss ascertainment	35 (33, 40)	68 (54, 78)	35 (30, 39)	61 (56, 65)	48 (37, 70)	<.001

Appendix E: Distribution of cycle day of positive pregnancy test



Cumulative incidence of positive pregnancy test by cycle day

Cycle day	Cumulative incidence
24	5%
24	10%
27	25%
29	50%
32	75%
37	90%
44	95%